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CONTRIBUTIONS FROM THE LABORATORY OF THE ROTHAMSTED EXPERIMENTAL STATION

(LAWES AGRICULTURAL TRUST)

REPRINTED FROM THE JOURNAL OF AGRICULTURAL SCIENCE, OCTOBER, 1909.

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THE EFFECT OF PARTIAL STERILISATION OF SOIL ON THE PRODUCTION OF PLANT FOOD.

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INTRODUCTION.

WHEN soil is partially sterilised, either by heat or by volatile antiseptics like carbon disulphide, toluene, etc., it becomes more productive and capable of yielding larger crops. The effect of heat was discovered incidentally about 25 years ago by the early soil bacteriologists; the action of carbon disulphide was first noticed somewhat later by a vine grower who had used it to kill phylloxera. Both cases have since been studied by several investigators, notably Koch[1] and Hiltner and Störmer[2]; a paper was also recently published by one of us[3] in which it was shown that the property is a general one, holding for all the soils and volatile antiseptics examined and for all the plants, excepting those of the leguminous order. Thus when a soil had been heated to 95° C. it produced two, three, or sometimes four times as much crop as a portion of the soil which had not been heated, whilst treatment with volatile antiseptics led to an increase in crop varying between 20 and 50 per cent. The treatment had in some way brought about a considerable increase in the amount of plant food—nitrogen, phosphorus, and potassium—obtainable by the plant; even more, indeed, than might be expected from the weight of the crop, since there was an increased percentage of nitrogen and phosphorus in the material of plants grown on the treated soils. The results quoted in

[1] Koch, *Arbeiten der deutschen Landwirtschaft-Gesellschaft*, 1899, Heft 40.

[2] Hiltner and Störmer, *Arbeiten der Biolog. Abteilung f. Land- u. Forstwirtschaft*, 1903, Bd. 3, Heft 5.

[3] Darbishire and Russell, *Journal of Agricultural Science*, 1908, Vol. II. p. 305. Full references to the literature of the subject are given in this paper.

the earlier paper were obtained with fertile soils, but we have obtained precisely similar results with an exhausted Rothamsted soil. (See Plate VIII, Figs. 1 and 2 and Table 1.)

Several hypotheses have been put forward to account for the increased productiveness. It was first supposed that a chemical reaction took place between the antiseptic and the soil whereby plant food was rendered more available; this view was soon discarded, but has recently been revived by Pickering[1]. Koch suggested a purely physiological hypothesis; the antiseptic was supposed to stimulate the plant roots to greater activity in extracting food from the soil. Such an action might have gone on in Koch's experiments where the antiseptic was left in the soil, but can hardly have taken place in ours, since all the antiseptic had been removed before the seeds were sown. Hiltner and Störmer attribute the action to the changed bacterial flora. They showed that the first effect of the antiseptic is to reduce the number of organisms, but when the conditions again became favourable the survivors multiply with extraordinary rapidity, and bring about a more intense production of nitrogenous plant food in the soil. They supposed that a larger amount of atmospheric nitrogen is "fixed," and the complex substances thus formed in the bacterial cells are slowly broken down to yield plant food. The decomposition processes normally taking place in the soil are probably hastened also, whilst the loss of nitrogen by denitrification is diminished. Other investigators have also supposed that increased nitrogen fixation is the main cause of the increased productiveness; on the other hand Koch[2] maintains that nitrogen fixation is decreased by partial sterilisation. Störmer[3] considers that the larger organisms are killed and decomposed by the surviving bacteria with production of ammonia. The dark green colour of the plants grown on partially sterilised soils has generally been regarded as an indication that the nitrogenous food stuff in the soil has in some way been increased by the treatment.

Part 1.

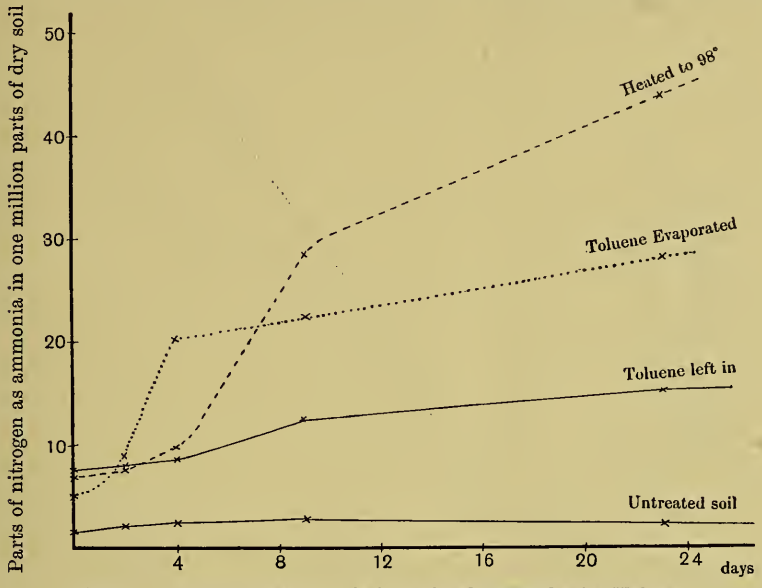
§ 1. We propose to give in this part a short statement of our experiments and the conclusions to which they lead, reference being made at each step to the paragraph in Part 2, where the full details and figures

[1] S. U. Pickering, *Journal of Agricultural Science*, 1909, Vol. III. p. 411.

[2] Koch, *Journal für Landwirtschaft*, 1907, Bd. 55, S. 355.

[3] Störmer, *Jahresber. d. Vereinigung für Angewandte Botanik*, 1907, S. 113.

are given. At the outset we may state that the soil employed in the experiments was taken from an arable field and contained moderate but not large amounts of nitrogen, organic matter, and calcium carbonate (§ 14). Partial sterilisation was effected either by heating to 98° C. or by addition of 4 per cent. of toluene, which at the end of three days was allowed to evaporate by spreading out the soil in a thin layer for as long as might be necessary. For convenience this soil is called "toluene evaporated" to distinguish it from a third series where the toluene was left in during the whole of the experimental period.



Curve 1. Amount of Ammonia in variously treated soils (Table 2).

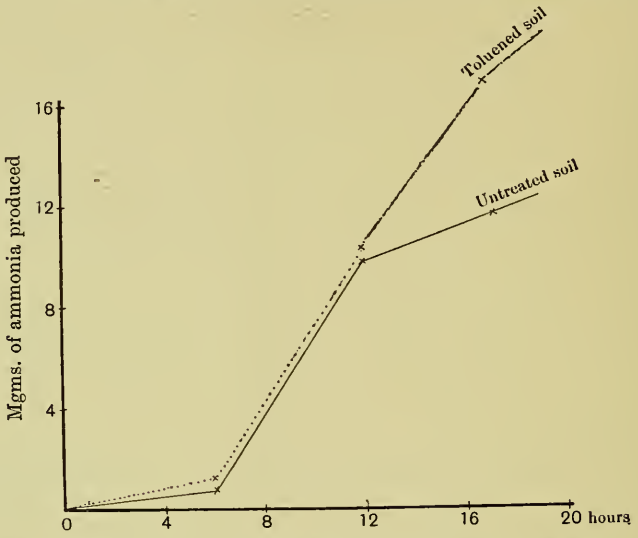
A fourth series consisted of untreated soils; a few experiments were also made with soils heated to 125° C., at which temperature all organisms are killed. After treatment the soils were moistened and kept for definite periods in bottles stopped with cotton-wool at the ordinary laboratory temperature. In these circumstances various changes soon set in, and are dealt with below.

I. *The changes taking place in partially sterilised soils.*

§ 2. (*a*) *Ammonia.* Curve 1 shows the amount of ammonia found in the various soils at stated intervals after the moisture had been added. In the untreated soil there is no accumulation of ammonia. The "toluene evaporated" soil and the soil heated to 98° C. show

that the treatment has effected a small immediate production of ammonia amounting to about 5 parts per million of soil, then little further change takes place for a few days. This period of comparative inaction is followed by one of rapid change, during which ammonia is produced in considerable quantity; lastly a slow period sets in, and the further production of ammonia is now only small. By the end of a month about 40 parts of nitrogen per million of soil have been converted into ammonia. (§ 15, Table 2.)

The difference between the "toluene evaporated" and the heated soils is only one of degree. The acceleration in the rate of formation of



Curve 2. Amount of ammonia produced from peptone solution after inoculation with arable soil (§ 24).

ammonia is evident at an earlier date in the toluened than in the heated soil, but is not maintained for so long, and by the ninth day the heated soil already contains more ammonia, a superiority which it maintains throughout.

The production of ammonia is mainly the work of micro-organisms. Proof is furnished by the following considerations:

1. The curves belong to the type associated with bacterial, rather than purely chemical change (cf. Curve 2).

2. Soil which has been heated to 125° C. (at which temperature all organisms are killed) behaves altogether differently: after the first production of ammonia due to heating there is no further change.

3. If the toluene is left in the soil there is only a slow production of ammonia and never a rapid rate; the curve is more nearly linear. The action of micro-organisms is here excluded, but enzymes may still act.

4. The rapid period sets in only when the soil is sufficiently moist. (Table 2.)

(*b*) *The production of unstable nitrogen compounds,* which may be regarded as intermediate products in the general decomposition, is also accelerated by partial sterilisation. (§ 17, Table 3.)

(*c*) *The humus,* on the other hand, appears to be but little affected; if anything there is a small increase, rather than a decrease, in the amount of humic nitrogen. It does not appear that the ammonia has been produced in the partially sterilised soils at the expense of humic nitrogen. (§ 19, Table 5.)

(*d*) *Nitrification.* The nitrifying organisms are destroyed by either method of partial sterilisation, but there is a very important difference between the two cases to which subsequent reference will be made. Toluene simply destroys the organisms: if they are again introduced after the toluene has been removed they at once begin to act. Heat not only destroys the organisms but brings about some change whereby the soil is rendered unsuitable for their development; they now no longer act even when re-introduced into the soil. (§ 31.) It appears that an inhibitory substance is formed by heat. References to Table 2 show that the untreated soil gains in nitrate whilst the toluened and heated soil do not.

(*e*) The change in the *total amount of nitrogen* is not great, even over a long period. There appears to be a small net loss from the partially sterilised soils as compared with the untreated soil: whether this result is due to diminished nitrogen fixation or to increased loss of nitrogen cannot be determined, but at any rate it disposes of the hypothesis that partial sterilisation is followed by an increase in the total nitrogenous matter in the soil. (§ 18, Table 4.)

§ 3. The two significant changes induced by partial sterilisation are thus seen to be (1) an increase in the amount of ammonia, (2) cessation of the nitrifying process.

The accumulation of ammonia which we have shown to take place in the treated soils is not simply due to the cessation of nitrification, for the amount of ammonia produced is greater than the sum of the ammonia and nitrate in the untreated soils after the same period. This accumulation may be due either (1) to an increased production of

ammonia in the treated soils, or (2) to the removal by the treatment of some agent, other than the nitrifying organisms, which is always consuming ammonia in the untreated soil. The second supposition falls to the ground, because when small quantities of ammonium salts are added to untreated soils the whole of the added nitrogen is recovered as ammonia and nitrate. (§ 21, Table 8.)

Hence we conclude that the treatment has induced an increased *production* of ammonia.

II. *The part played by bacteria.*

§ 4. We have confirmed Hiltner and Störmer's discovery that bacteria multiply more rapidly and reach far higher numbers in the partially sterilised than in the untreated soils. Our untreated soils usually contained about 5 to 9 million organisms per gram, a number which remained fairly constant. Treatment with toluene effected a considerable reduction, but subsequently, when the toluene had gone and moisture was added, a period of rapid multiplication set in, and the numbers rose to 40 millions or more. The numbers of bacteria increase *pari passu* with ammonia production, and we may therefore associate the increased ammonia production with the increased numbers of bacteria. (§ 22, Table 9.)

§ 5. Examining this conclusion in some detail, there is no evidence that the species surviving the treatment have received a stimulus which makes them more active or that they are the more active survivors of a mixed race. The contrary is rather the case; for instance, *B. mycoides*, and the brown and white streptothrix, were isolated from the toluened soil, and all proved less active than the same organisms obtained from the untreated soil. (§ 26.) On the other hand we obtained considerable evidence that the whole surviving flora is more active than the original one in effecting the decomposition of nitrogenous organic substances such as peptone, etc., and in hydrolysing urea. (§§ 24 and 27, Table 10.)

Not only is the whole soil with its flora more active, but also the flora carried in an aqueous extract of the soil. (§ 25.) The extract is prepared at ordinary temperatures by shaking soil with water and filtering through cotton-wool; it contains all organisms that are readily detached from the soil and sufficiently small to pass through the filter. Such an extract prepared from the partially sterilised soils proved more active than extracts of untreated soil in decomposing peptone.

§ 6. Examination of gelatine plates prepared by Koch's method shows that the flora which establishes itself in the soil after heating is altogether different from that originally present, but, on the other hand, the flora of the toluened soil did not appear to have markedly altered. It is true that certain species were completely suppressed by toluene, but their number was only small: indeed out of 27 found in the untreated soil only three failed to appear in the toluened soil. Of these the most striking is a fluorescent organism, which however did not appear to influence the changes one way or the other. (§ 36.) Further, of the two streptothrix varieties, the brown predominated in the untreated soil and the white in the toluened, but their difference does not appear to be significant. (§ 26.) The curves for ammonia production in the heated and toluened soil (Curve 1) are very much alike, whilst the bacterial flora is very different: the curves for ammonia production in the toluened and untreated soil are fundamentally different, whilst the bacterial flora is not. We cannot therefore attribute the difference in the rate of ammonia production to a change in the type of bacterial flora.

Our experiments indicate that the increased ammonia production in the partially sterilised soil is due to the increased numbers of the bacteria. The problem reduces itself to finding out why the bacteria can increase so much more rapidly in the partially sterilised, than in the untreated soils.

§ 7. Further evidence that the comparative inertness of the bacteria in the untreated soil cannot be caused by any bacterial factor is afforded by the following considerations:

(*a*) If a filtered soil extract containing bacteria from an untreated soil is added to a toluened soil there is an increase in the rate of ammonia production, and also in the number of bacteria.

(*b*) But if untreated soil is added to toluened soil there is no increase in the rates of ammonia production or of bacterial multiplication, but, on the contrary, a reduction. These results are set out on Curve 3, Table 13, § 36.

(*c*) As pointed out above, an extract of toluened soil is more active than an extract of untreated soil.

(*d*) But when the extract of toluened soil is added to the untreated soil there is no increase in ammonia production.

The conclusion may be drawn that *the untreated soil contains a factor, not bacterial, limiting the development of bacteria, this factor being put out of action by toluening or heating.*

III. *The nature of the limiting factor.*

§ 8. The limiting factor is not a toxin such as are postulated by Whitney and others.

(1) If it were, it would be sure to affect the nitrification bacteria most as they are more sensitive than the ammonia producing groups, as seen :

(*a*) in their absence from toluened or heated soils,

(*b*) in the fact that they cannot be reintroduced into a heated soil because the heating has developed some substance toxic to nitrifying organisms, but not to ammonia producing organisms. (§ 31.)

In the untreated soil nitrates but never ammonia accumulate, and the rate of nitrification is at least as great as the rate of ammonia production. If there is nothing toxic to the nitrifying organisms, *a fortiori*, it is very unlikely there is anything toxic to the ammonia producers.

(2) Barley seedlings grown in aqueous extracts of untreated and toluened soils with or without addition of culture solution showed no difference in growth over a period of four weeks. Had any toxin been present it should according to Whitney have produced an effect in much less time.

§ 9. The limiting factor is probably biological, since when untreated soil is added to toluened soil the reduction in the rate of ammonia production is not at once operative. (Curve 3 (p. 140), § 36.) It is probably also a large organism, since it is only the soil and not the filtered extract of the untreated soil that is effective in reducing the rate of ammonia production in toluened soil. (Cf. § 7*a*, also §§ 38 and 39.) Search was therefore made for large organisms such as infusoria, amoebae, and ciliata. None were found in the heated soil, and only small ciliate infusoria in the toluened soil. All these organisms are found in the untreated soil. Some, *e.g. Colpoda cucullus* and *Amoeba nitrophila*, are known to devour bacteria, and all must be severe competitors by reason of their large size (about 1000 times that of the soil bacteria). We conclude then that *these large organisms—protozoa, etc.—constitute the factor, or one of the factors* (see § 42) *limiting the bacterial activity, and therefore the fertility of our untreated soil.* Direct evidence is furnished by inoculating toluened soil or soil extract with cultures of large organisms, and studying the effect produced. Curve 4 shows the consequent depression in the rate of ammonia formation. (§ 39, Table 14.)

§ 10. We are now in a position to account for all the changes brought about by partial sterilisation.

The micro-organic flora of the ordinary arable soil with which we started is very mixed, and includes a wide variety of organisms performing very different functions. For our purpose they may be divided roughly into two classes: saprophytes, which live on and effect the decomposition of organic matter and a class comprising

(*a*) phagocytes which consume actual living bacteria,

(*b*) large organisms inimical in other ways to bacteria.

The action of the saprophytes tends to increase the fertility of the soil, *e.g.* they produce ammonia, fix nitrogen, and so on. It is true that some of them bring about liberation of free nitrogen during the decomposition of organic matter, and are to this extent injurious, but such action is either much restricted, or is counterbalanced by the fixation process, and does not affect our general statement. The phagocytes, and similar organisms, on the other hand, must be detrimental to fertility because they limit the number of the organisms and therefore the rate of ammonia production.

Between these two classes of organisms there is an equilibrium under natural conditions; the bacteria cannot multiply indefinitely, but are kept in check by the phagocytes; the phagocytes, on the other hand, are kept in check by the limited amount of food, and no doubt also by other adverse conditions, such as lack of water[1]. In these circumstances bacteria effect only a limited amount of decomposition, much less, in fact, than might be expected from the total amount of organic matter present.

When toluene is added, or when the soil is heated to 98°, the phagocytes are killed, but the bacterial spores are not. On removing the toluene and adding moisture, the spores germinate and the resulting organisms multiply with great rapidity, since they are now freed from the attacks of their enemies and the competition of other large organisms; they even appear to decompose the dead organisms. There is evidence to show that the individual species may be less virulent than the old races; but they more than make up for any deficiency in this direction by their enormously increased numbers. The rate of decomposition is considerably hastened, and a largely increased amount of ammonia is produced. Some of the groups of organisms suffer, such as the

[1] On this view it is easy to explain Rahn's results, which have hitherto remained very obscure. He found that drying the soil at ordinary temperature increased its productiveness but did not cause what he considered sufficient alteration in the bacterial flora or the food supply (*i.e.* the *immediate* food supply), *Centr. für Bakteriologie*, 1908, II. Bd. 20, S. 38.

nitrogen fixers (§ 29), whilst the nitrifying organisms are absolutely exterminated.

It might be thought that the removal of nitrifying organisms would seriously interfere with the growth of plants, but, as a matter of fact, it seems to have but little effect; plants readily take up the decomposition products—ammonia, etc. Nitrification is shown to be economical, but not essential. (§ 44.) The excess of nitrogenous plant food in the partially sterilised soil soon becomes so great that it causes a correspondingly vigorous plant growth.

§ 11. Partial sterilisation has been found to increase fertility on many types of soil and always by increasing the supply of nitrogenous plant food. There is reason to suppose therefore that the large destructive and competing organisms will be found of common occurrence on ordinary soils, checking the beneficent bacteria and limiting fertility. An important practical problem arises: is it possible to suppress them in ordinary field soils by any economical and practical process? This problem is under investigation. It is unnecessary at this stage to enlarge on the importance both from the practical and scientific point of view of these large organisms as factors in soil fertility. A fuller study of them will no doubt throw much light on many soil problems, at present obscure. We are now engaged in further investigations of these organisms.

§ 12. Our results may be summarised as follows:

(1) The increased productiveness of partially sterilised soils is due to an increase in the amount of ammonia present.

(2) The excess of ammonia is the result of increased decomposition of soil substances by bacteria.

(3) Hiltner and Störmer's discovery that the bacteria increase rapidly after partial sterilisation, and finally become much more numerous than in the original, untreated soil, is confirmed. The increase in number proceeds *pari passu* with the increase in ammonia.

(4) The new bacterial flora arising after partial sterilisation is a more potent decomposing agent than the original flora, but the individual species have not become more, but apparently less potent. The increased decomposing power of the new flora is associated with its numerical superiority over the old flora.

(5) The rates of decomposition and of bacterial increase in the toluened soil were found to be adversely affected by the addition of the original untreated soil. The original soil therefore contains some factor which limits bacterial action.

(6) Chemical hypothesis having been found unsatisfactory the

factor is shown to be biological. Large organisms (protozoa) were found in the untreated, but not in the partially sterilised soils, at least two of which are known to destroy bacteria.

(7) These large competing and destructive organisms are killed by heat and most of them by toluene, and can then serve as food for bacteria. In both these directions the effect of partial sterilisation is beneficial.

(8) As the effect of partial sterilisation in increasing productiveness is shown on so many soils, and apparently always in the same way, it may be expected that these competing and destructive protozoa are of common occurrence and constitute an important factor in soil fertility.

(9) In relation to plant growth partially sterilised soils are peculiar in that they supply not nitrate, but other nitrogen compounds such as ammonia, to the plant. The nitrifying organisms will develope if they get into the toluened soil, but they did not work in our heated soils. With this difference in the course of nitrogen nutrition may be correlated the difference in nitrogen content of the plant and in the character of growth.

Part 2.

Experimental.

§ 13. *Crop results.* The soil was taken from the outside strip of Barnfield, and had been unmanured for many years. It was brought down in quantity to the plant-house, spread on a clean cement floor, sieved, and carefully picked over to remove worms. The picking over requires great care and is very laborious; unless it is properly done the crop weights in duplicate experiments are likely to be discordant. The soil was next weighed into pots, tipped out, and mixed with 10 per cent. of sand; then it was either replaced, heated to 98° C. in a large steam oven, or treated with toluene, according as it was to be an "untreated," a "heated," or a "toluened" soil. In the latter case 2 c.c. of toluene were added for every kilogram of soil, left to act for three days, then allowed to evaporate by spreading the soil out in a thin layer for a sufficient length of time. Finally all the pots were weighed, and water added till 18 per cent. was present, an amount which was kept fairly constant throughout the period of plant growth.

The results obtained with successive crops are given in Table 1.

TABLE 1. *Crop results obtained on partially sterilised soils.*

1*st crop, Rye.*

Soil	Weight of green crop	Weight of dry matter		Composition of dry matter			Weight of material taken from soil		
		In grams	Relative weights	N per cent.	P_2O_5 per cent.	K_2O per cent.	N grams	P_2O_5 grams	K_2O grams
Untreated...	103·95	37·14	100	·698	·59	1·05	·259	0·22	0·39
Heated	162·10	59·30	160	1·147	·64	1·28	·680	0·38	0·76
Toluened ...	120·07	44·76	120	·742	·54	1·01	·332	0·24	0·45
2*nd crop, Buckwheat.*									
Untreated...	44·34	8·53	100	1·179	1·22	2·22	·101	·10	·18
Heated	56·07	11·19	131	1·270	1·34	2·05	·142	·12	·22
Toluened ...	40·56	7·79	91	1·166	1·47	2·09	·104	·11	·16

The treatment was then repeated, and wheat was grown. The crop is still standing, but the differences are of the same order as those obtained with rye.

Plate VIII, Fig. 1 shows the rye and Plate VIII, Fig. 2 the wheat crops. On reference to the earlier paper it will be found that these results, obtained on an exhausted soil, are very similar to those obtained on fertile soils. The crops grown on heated soils are shorter in the straw and more compact than the others; typical internodal lengths in cms. are:

Number of internode, counting from soil.....	1st	2nd	3rd	4th	5th	6th	Length of ear
Rye grown on untreated soil ...	5·5	14	21·5	27	38	51	11
,, toluened ,, ...	7	15	20	21·5	29	36	11
,, heated ,, ...	1	11	16	23	28·5	34	11

The chemical changes taking place after partial sterilisation.

§ 14. Two soils have been used in these experiments: the Barnfield soil, alluded to above, and a soil from the outside strip of Little Hoos which had until 1902 been in ordinary arable cultivation, and since that year has carried the same crop as the rest of the field, but has had occasional dressings of artificial manures. The two soils contained:

	Nitrogen	Loss on ignition	$CaCO_3$
Barnfield	·112 %	3·969 %	3·409 %
Little Hoos Field ...	·178 %	4·572 %	3·159 %

The chemical investigation was directed mainly to the nitrogen compounds, ammonia, nitrates, complex and unstable nitrogen compounds, and humus. The soil was picked over, passed through a 3 mm. sieve, and put up in quantities of 800 grams into large bottles. These were then divided into four sets: to each bottle in one set was added 40 grams of toluene, which was allowed to remain in the soil during the whole of the experimental period; a second set also received the same amount of toluene, but at the end of three days the soil was spread out till the toluene evaporated; a third set was heated to 98° C. for 3 hours, and the fourth was left untreated as control. After these various treatments the water content of the soil was brought up to 15 per cent. Precautions were taken to prevent reinfection, and the bottles were kept plugged with cotton-wool to admit air; they were stored in a cupboard at about 15° C. At suitable intervals a bottle was taken from each set and the determinations above referred to were made.

§ 15. *Changes in the amount of ammonia and of nitrate present.* The determination of ammonia in soils is complicated by two factors which, however, act in opposite directions: soil has a remarkable power of retaining ammonia, and, on the other hand, some of the organic compounds of the soil are very unstable and readily decompose with formation of ammonia. By distilling soil at 12 mm. pressure with water containing 2 per cent. of magnesia in suspension we have succeeded in reducing these sources of error and obtaining quite satisfactory results.

Although ammonia is the final nitrogenous product of the decomposition of organic matter in the soil it is not the final result of the bacterial activity in the untreated soil, but is at once changed to nitrate. It is therefore necessary to make simultaneous determinations of ammonia and of nitrates.

The results of two of the experiments are given in Table 2. Soil 1 had two years previously received a complete dressing of artificial manure, whilst Soil 2 had been for some years unmanured.

TABLE 2. *Ammonia and nitrate present in partially sterilised soils, expressed as parts of nitrogen per million of soil dry at 100° C.*

Soil 1. 15 per cent. of water.

Treatment of soil	Nitrogen present as ammonia					Nitrogen present as nitrate			
	At beginning	After 7 days	After 15 days	After 31 days	After 150 days	At beginning	After 15 days	After 31 days	After 150 days
Untreated	2·2	1·9	2·7	2·2	8·3	17	24	26	33
Heated to 98° C.	8·6	27·0	32·6	37·0	83·0	17	16	13	17
Toluene evaporated	4·2	27·9	34·3	34·6	nil*	15	16	13	73
Toluene left in	4·2	—	—	7·5	—	18	—	16	—

* Infected with the nitrifying organism.

Treatment of soil	Total nitrogen as nitrate and ammonia				
	At beginning	After 31 days	After 150 days	Gain in 31 days	Gain in 150 days
Untreated	19·2	28·2	41·3	9	22·1
Heated to 98° C.	25·6	50·0	100	24·4	74·4
Toluene evaporated	19·2	47·6	73	28·4	53·8
Toluene left in	22·2	23·5	—	1	—

Soil 2. 1st period: 8 per cent. of water present.

Treatment of soil	Nitrogen present as ammonia			Nitrogen present as nitrate		Total nitrogen as ammonia and nitrate		
	At beginning	After 13 days	After 63 days	At beginning	After 13 days	At beginning	After 13 days	Gain in 13 days
Untreated	1·3	1·8	1·7	13	12	14·3	13·8	nil
Heated to 98° C.	5·0	6·5	13·1	15	12	20	18·5	−1·5
Toluene evaporated	4·0	5·0	14·5	13	13	17	18	1
Toluene left in	4·3	7·2	20·7	12	13	16·3	20·2	3·9

2nd period: 17 per cent. of water present.

Treatment of soil	Nitrogen present as ammonia						Nitrogen present as nitrate		Total nitrogen as ammonia and nitrate		
	At beginning	After 2 days	After 4 days	After 9 days	After 23 days	After 54 days	At beginning	After 23 days	At beginning	After 23 days	Gain in 23 days
Untreated	1·8	2·0	2·2	2·5	1·7	trace	12	16	13·8	17·7	3·9
Heated to 98° C.	6·5	7·5	9·7	28·1	43·8	46·9	13	12	19·5	55·8	36·3
Toluene evaporated	5·0	8·9	20·0	22·1	27·8	34·3	12	12	17·0	39·8	22·8
Toluene left in	7·2	6·7	8·5	12·7	14·5	19·4	11	10	18·2	24·5	6·3

The results for Soil 2 are plotted on Curve 1 (p. 113).

§ 16. The immediate effect of heating the soil to 95° C. or of treating with toluene is to cause a small production of ammonia amounting to about 3 to 5 parts per million of soil, and on standing there is a further production, the extent of which depends on the amount of water present. With 8 per cent. of water the action is slow and the curve is linear, but when 17 per cent. is present the curve characteristic of bacterial processes is obtained. Action is slow for a few days, but by the ninth day it has become very vigorous and remains so for a time; then it slackens considerably. Soil which has been heated to 125° C. (at which temperature all organisms are killed) behaves altogether differently: after the first production of ammonia due to heating there is no subsequent change. It is clear then that the continuous formation of ammonia in the partially sterilised soil is due to living organisms.

Where toluene is left in the change is quite different; even with 17 per cent. of water the curve is nearly linear. The action of micro-organisms is in this case excluded, but any enzymes set free could continue to bring about decomposition.

The untreated soil differs from all the others; there is no accumulation of ammonia either with 8 or 17 per cent. of water, but there is an increase in the amount of nitrate, and the sum of ammonia- and nitrate-nitrogen shows a small gain amounting to 9 parts per million in 31 days in Soil 1, and 4 parts per million in 23 days in Soil 2. It is known that the nitrifying organisms only produce nitrates from ammonia; these quantities therefore indicate ammonia that has been formed and then nitrified.

§ 17. *Unstable nitrogen compounds.* When soil is boiled at ordinary pressure with water containing magnesia in suspension there is a steady and continuous evolution of ammonia arising from the decompositon of unstable nitrogen compounds. By working under definite conditions it is possible to obtain comparable results; determinations made simultaneously with those recorded in Table 2 are set out below. The immediate effect of toluene and of heat is to increase the unstable nitrogen compounds, and is therefore something more than a simple liberation of ammonia: there is not however as great a subsequent accumulation of the unstable compounds.

§ 18. *Total nitrogen.* The net change in the amount of nitrogen has alone been investigated: it is not at present possible to measure the separate processes of fixation and loss. In order to make the

TABLE 3. *Quantities of ammonia liberated at* 100° *and at* 38°; *parts per million of dry soil.*

	Untreated soil				Soil heated to 95°				Toluene evaporated				Toluene left in			
	At beginning	After 9 days	After 23 days	After 54 days	At beginning	After 9 days	After 23 days	After 54 days	At beginning	After 9 days	After 23 days	After 54 days	At beginning	After 9 days	After 23 days	After 54 days
Liberated at 100°	8·9	6·3	6·6	5·8	16·8	43·5	61·6	76·2	20·7	37·8	45·0	55·7	19·6	23·1	22·1	28·8
,, 38°	1·8	2·5	1·7	trace	6·5	28·1	43·8	46·9	5·0	22·1	27·8	34·3	7·2	12·7	14·5	19·4
Difference ("unstable nitrogen compounds")	7·1	3·8	4·9	5·7	10·3	15·4	17·8	29·3	15·7	15·7	17·2	21·4	12·4	10·4	7·6	9·4
Gain in 54 days:—Unstable compounds			−1·4				19·0				5·7				−3·0	
Ammonia			−1·8				40·4				29·3				12·2	

TABLE 4. *Changes in total nitrogen.*

	Soil 1, kept 15 months			Soil 2, kept 10 months			Soil 3, kept 7 months		
	N at end of period, per cent.		Difference from untreated	N at end of period, per cent.		Difference from untreated	N at end of period, per cent.		Difference from untreated
Untreated soil	·1106 ·1106	Mean ·1106		·1143 ·1154	Mean ·1149		·1825 ·1828	Mean ·1827	
Soil heated to 98°	·1124 ·1159	·1142	+ ·0036	·1068 ·1023	·1046	− ·0103	·1788 ·1775	·1782	− ·0045
Toluene evaporated	·1093 ·1110	·1102	− ·0004	·1114 ·1083	·1099	− ·0050	·1741 ·1720	·1731	− ·0096

difference as large as possible the soils were kept for some months: the results are given in Table 4.

When Soil 1 was put up it contained ·1196 per cent. of nitrogen; during the 15 months the untreated soil has lost ·009 per cent. In no case does the toluened soil contain more nitrogen than the untreated: the assumption that the increased productiveness of partially sterilised soils is due to increased nitrogen fixation is therefore wrong. On the contrary there appears to be an actually greater loss of nitrogen from the toluened soil, and in two cases also from the heated soil than from the untreated soil.

§ 19. *Humus.* Since humus is a somewhat indefinite group of substances the method of determinations must be arbitrary, but by working under definite conditions it is possible to get comparable results. The soil is washed with dilute hydrochloric acid till the washings are free from calcium, then with water, finally it is shaken with a 4 per cent. solution of ammonia to dissolve the humus. Determinations are made of the total organic matter in the extract (humus), and of nitrogen left after the ammonia has been boiled off (humic nitrogen).

Table 5. *Changes in humus.*

Soil 1, kept 15 months.

	Humus, per cent. in soil	Humic nitrogen, per cent. in soil	Nitrogen in humus, per cent.
Amount originally present	1·06	·047	4·4
After 15 months, untreated soil...	·91	·049	5·4
Soil heated to 98°	·93	·043	4·7
Toluene evaporated ...	·90	·051	5·6

The total amount of nitrogen is given in Table 4, Soil 1.

Soil 2, kept 10 months.

	Humus, per cent. in soil	Humic nitrogen, per cent. in soil	Nitrogen in humus, per cent.
Untreated soil	·90	·046	5·1
Soil heated to 98°	·82	·038	4·6
Toluene evaporated	·86	·048	5·6

The experimental error is rather large, and too much stress must not be laid upon small differences, but so far as the figures have any

significance they show that the toluened soil loses a little more humus than the untreated, but gains a little more humic nitrogen. It is, however, quite certain that the toluened soil has not lost any humic nitrogen, and the increased ammonia and nitrate recorded in Table 2 cannot have come from humic nitrogen.

The heated soil behaves rather differently from the others, but we have obtained a good deal of evidence to show that heat decomposes humus, and brings about a loss of humic nitrogen.

The distribution of nitrogen compounds in a typical case is as follows:

TABLE 6.

	Nitrogen in parts per million of soil					
	Nitrate	Ammonia	Unstable* compounds	Humic compounds	Other compounds (by difference)	Total
Untreated soil at beginning ...	12	1·8	7·1	840	779	1640
,, after 23 days...	16	1·7	4·9	842	769	1634
Heated soil at beginning	13	6·5	10·3	600	1010	1640
,, after 23 days	12	43·8	17·8	—	—	1640
Toluened soil at beginning ...	12	5·0	14·7	840	768	1640
,, after 23 days ...	12	27·8	17·2	846	727	1630
Gain in untreated soil	4	0	−2·3	2	−10	− 6
,, heated soil.....	−1	37·3	7·5	—	—	0
,, toluened soil.....	0	22·8	2·5	6	−41	−10

* The "unstable compounds" merge into "other compounds," and the division line is purely arbitrary.

§ 20. *Absorption of oxygen.* This was investigated by the method devised by one of us and described elsewhere[1]. The relative amounts absorbed in 10 days by the various soils are given below and show that the toluened and heated soils absorb during the first month more than does the untreated soil. This result has been previously obtained and appears to be quite general. After a time, however, the rate of absorption begins to fall off and at the end of 72 days both the toluened and the heated soils are absorbing less than the untreated soil.

[1] E. J. Russell, this *Journal*, 1905, Vol. I. p. 261.

Table 7. *Relative absorption of oxygen in* 10 *days: untreated soil* = 100.

	Untreated soil	Heated soil	Toluened soil
At beginning	100	100	206
After 28 days.....	87	145	102
After 72 days.....	63	25	51

§ 21. The increased amount of ammonia in the partially sterilised soil is not in itself a sufficient proof of increased ammonia production: it might equally arise from a diminished ammonia assimilation if we assume the presence of some ammonia or nitrate consuming organism in the untreated soil, but not in those partially sterilised. We failed, however, to find any evidence of such a process. Periodical determinations of the ammonia and nitrate in soils to which known quantities of ammonium sulphate had been added always accounted for all or more than all of the added ammonia. Some of the results obtained are as follows:

Table 8. *Effect of adding ammonium sulphate to soil.*

	Nitrogen present as ammonia				Nitrogen present as nitrate				Total nitrogen as ammonia and nitrate		
	At beginning	After 6 days	After 13 days	After 55 days	At beginning	After 6 days	After 13 days	After 55 days	At beginning	After 55 days	Difference
Untreated soil ...	122·8	111·0	91·3	4·9	18·5	18·7	40·0	157·2	141·3	162·1	+20·8

These experiments show that (1) the increased productiveness of a partially sterilised soil is due to an increase in the amount of ammonia present,

(2) the increased amount of ammonia is the result of bacterial action,

(3) it is not due to a diminished assimilation of ammonia or nitrate but to an actual increase in the rate of ammonia production.

§ 22. *The total number of bacteria capable of developing on gelatine plates.* The first effect of partial sterilisation is to reduce considerably the number of these organisms present, but when the soil is subsequently

moistened an enormous increase takes place. This fact was first observed by Hiltner and Störmer and has been repeatedly confirmed during the course of our investigations; as an instance the following figures may be quoted:

TABLE 9.

Soil 1. Arable soil from Little Hoos Field (§ 14).

	Number of organisms per gram of dry soil			Ammonia produced in 9 days, parts per million of dry soil
	At beginning	After 9 days	Increase during 9 days	
Untreated soil	6,693,000	9,814,000	3,121,000	0·7
Toluene evaporated	2,608,000	40,620,000	38,012,000	17·1
Heated soil	393	6,294,100*	6,294,000*	3·2*
Toluene left in	2,311,000	2,617,000	300,000	5·5

* After 4 days, 9 days' counts lost by plates liquefying.

Soil 2. Rich garden soil containing ·592 per cent. of nitrogen.

	At beginning	After 10 days	After 38 days
Untreated soil	4,200,000	10,600,000	13,850,000
Toluene evaporated...	1,306,000	31,680,000	38,200,000
Heated soil	40	7,360,000	17,600,000

Plate IX, Figs. 4 and 5 show the photographs of the plates.

The rapid rate of increase in the partially sterilised soils is not maintained indefinitely, but even after some months the toluened soil contains more organisms than the untreated soil, the actual excess depending on the conditions that have obtained in the meantime.

A very important relationship is brought out by the above figures. It will be noticed that the increase in the number of organisms runs parallel with the increase in the amount of ammonia: we may therefore infer that the increased ammonia production is associated with the increase in the number of bacteria.

§ 23. *The production of ammonia by soil bacteria from nitrogenous compounds.* For the elucidation of this problem we had recourse in the first instance to a method devised by Remy which, after suitable modifications, gave very useful results.

§ 24. *The production of ammonia from peptone.* In Remy's method 10 grains of soil are inoculated into 100 c.c. of a culture solution containing 1 per cent. of peptone besides nutrient salts, but no ammonia,

and the whole is left for three days in an incubator. The ammonia produced is then determined. In most of our experiments made in this way inoculation with toluened soil caused about 15 per cent. greater production of ammonia than inoculation with untreated soil, whilst heated soil only yielded about half as much: the results however were not always consistent and it sometimes happened that the toluened soil culture gave no more ammonia than the untreated. After numerous trials three modifications were finally introduced.

(1) Instead of stopping the reaction at any arbitrary moment and expressing the result as a number we have found it better to make a series of determinations at definite intervals and plot the results as a curve expressing the rate at which reaction takes place. In this way the method becomes more sensitive and gives more useful information.

(2) The cotton-wool plug was replaced by an acid trap to prevent loss of ammonia by volatilisation.

(3) A stronger inoculation was made, 25 grains of soil being introduced into 15 c.c. of a 3·3 per cent. solution of peptone.

Of these the third is purely a matter of convenience; the general type of curve obtained is independent of the strength of inoculation. Two experiments made by the modified method are recorded below: the figures are plotted on Curve 2 (p. 114).

Experiment 1. Arable soil as used for determinations given in Table 2.

Treatment	Ammonia (expressed as nitrogen) in mgrams produced after			
	6 hours	12 hours	18 hours	24 hours
Untreated soil	·8	9·8	11·5	26·3
Soil heated to 98°.....	0	0	0	2·7
Toluened soil.....	1·2	10·1	17·3	27·7

The number of organisms in the culture was determined by nutrient agar plate culture: the results, expressed as the number per gram of soil present, are:

Treatment	At beginning	After 6 hours	After 12 hours	After 18 hours	After 24 hours
Untreated soil	28,900,000	100,500,000	866,000,000	933,000,000	1,310,000,000
Soil heated to 98°...	1,000	60,200	814,000	5,280,000	9,500,000
Toluened soil.....	2,520,000	11,700,000	40,500,000	66,400,000	128,800,000

Repetition of the experiment with another soil and with gelatine plates gave similar results. The plates showed sharp differences in flora; fluorescent bacteria predominated on the plates poured from the untreated soil cultures but were absent from the others: *B. mycoides* and *zopfii* were most numerous on the plates poured from the toluened soil. It is shown later on that *B. mycoides* decomposes peptone much more rapidly than *B. fluorescens.*

Experiment 2. A rich garden soil.

Treatment	Ammonia in mgrams produced after						
	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours	30 hours
Untreated soil ...	·9	2·9	8·8	15·0	25·5	30·1	39·9
Heated soil	·5	·7	2·9	5·0	6·2	8·5	15·0
Toluened soil ...	2·0	6·0	15·4	23·9	33·6	35·3	42·6

This rate of change is at first slow, then it rapidly increases and finally it slackens. The rapid period sets in some time earlier in the toluened soil than in the untreated, but is delayed considerably in the heated soil probably because of the small number of organisms which survive a temperature of 98° C.

It was found that a little toluene reduced the decomposition rate almost to zero, thus affording further proof, if more were needed, of the bacterial origin of the change.

§ 25. It is by no means necessary that soil should be used as the inoculating material in these experiments. The filtered liquid from the peptone cultures readily decomposes peptone, and in this case also the culture obtained from the toluened soil is more potent than that from the untreated.

Filtrate from	Ammonia in mgrams produced after			
	8 hours	16 hours	24 hours	36 hours
Untreated soil culture.....	0·1	2·7	5·2	8·1
Toluened soil culture	0·6	1·5	6·2	11·3

Again, the extracts obtained by shaking some of the soil with water and filtering through cotton-wool show the same kind of difference in

the amount of decomposition they bring about when inoculated into peptone solution. The bacteria present in the extract of the toluened soil are more effective than those in the extract of the untreated soil; the results are:

Extract from	Ammonia in mgrams produced after		
	12 hours	18 hours	32 hours
Untreated soil ...	1·7	3·8	10·8
Toluened soil.....	2·1	4·2	16·6

Similar results are obtained when other nitrogenous compounds are substituted for peptone. Casein, gelatine and lucerne hay infusion were all decomposed more readily by the toluened than by the untreated soil.

It is thus clear that the flora which survives the process of partial sterilisation and developes when the conditions again become favourable is more effective in producing ammonia from complex nitrogen compounds than the original flora of the soil.

§ 26. This conclusion however only applies to the community of organisms considered as a whole. If we isolate any individual species of organism we find that the cultures made from the toluened soils are actually less potent than cultures of the same organism from untreated soils. The amounts (in mgms.) of ammonia produced from peptone solution in 76 hours were found to be:

Organisms from	*B. mycoides*	White streptothrix	Brown streptothrix
Untreated soil	10·2	2·5	2·6
Heated soil	5·9	2·0	2·2

It is clear that we must not explain the effects of partial sterilisation by assuming that the separate organisms or group of organisms are rendered more virulent, or more effective by loss of weaker members, or that they are stimulated for any long period by the temporary action of the toluene. The contrary indeed happens, and the individual species rather suffer by the treatment. An interesting point brought out is that the brown and white streptothrix possess very similar decomposing powers.

§ 27. *The production of ammonia from urea.* The experiments with urea were on the same lines as the preceding and lead to the same conclusions.

TABLE 10.

Treatment of soil	mgrams of ammonia produced after				Bacteria present in millions per gram of soil				
	8 hours	16 hrs.	24 hrs.	32 hrs.	0 hour	8 hours	16 hrs.	24 hrs.	32 hrs.
Untreated.....	1·7	3·9	5·9	6·4	2·4	2·1	3·0	7·0	1·5
Toluene evaporated..	2·8	8·3	16·5	57·4	·78	3·0	5·9	13·0	66·0
Toluene left in	0	2·1	4·3	5·3	2·0	2·1	2·8	2·7	2·1
Heated to 98° C.....	0	0	2·5	0		·1		·9	1·7
Heated to 120° C. ...	0	0	1·4	0	0	0	0		0

Decomposition has been most rapid in the "toluene evaporated" soil but it also goes on in presence of toluene. It is not due in the latter case to catalytic action of the soil since it does not take place in the heated soils, but is most probably brought about by an enzyme.

§ 28. There is a fundamental difference between the decomposition of peptone and of urea. Peptone acts as a nutrient, urea does not, but is decomposed by a purely fermentative change. The aqueous extract of the soils had little or no action on urea solution till peptone was added and then decomposition took place: the amount of decomposition increased with the amount of peptone added.

TABLE 11. *Effect of nitrogenous food supply on the rate of urea hydrolysis* (*25 grams untreated soil and 15 c.c. 1 °/₀ urea solution and varying amounts of nitrogen as peptone*).

Nitrogen added as peptone, mgrams	0	0·26	0·52	1·04	2·08
Mgrams of ammonia, expressed as nitrogen, produced after 44 hours	6·6	8·0	13·0	31·9	59·0

Under the same conditions toluened soil produced 53·8 mgms. of nitrogen as ammonia; a striking proof of its superior decomposing powers.

§ 29. *Nitrogen fixation.* 5 grams of soil were inoculated into 50 c.c. of a 2 per cent. mannite solution containing potassium phosphate (Beyerinck's solution) and the whole was allowed to stand at 30° in an Erlenmeyer flask plugged with cotton-wool for 21 days. As no nitrogen compound was supplied those organisms alone could develope that take their nitrogen direct from the air. The toluened soil fixed less than the untreated, whilst the heated soil fixed practically none.

The actual amount of nitrogen fixed per gram of mannite supplied was:

	Arable soil	Garden soil
Untreated soil	4·7	6·3 mgrams
Toluened soil	3·8	6·2 ,,
Heated soil	·5	·2 ,,

These results confirm our previous conclusion that the increased productiveness of partially sterilised soils is not due to increased nitrogen fixation.

§ 30. *Nitrification.* Both heat and toluene destroyed the nitrifying organisms; there was no sign of revival even after a month's incubation at 30°. We have already shown (Table 2) that nitrates are not produced in partially sterilised soils except as a result of subsequent infection.

It has of course been known for many years that volatile antiseptics put an end to nitrification, but it is usually considered that the nitrifying organisms recover after an interval, and even, according to some, work at an increased rate.

We have made a number of experiments on this point, but in no instance have we obtained any evidence of recovery when sufficient precautions were taken to guard against re-infection. It was a common experience that nitrification would be for a long time suspended in toluened soils and would then set in with the production of a large amount of nitrate, thus:

	At beginning	After 6 weeks	After 18 weeks
Parts of nitrogen as nitrate per million of dry soil	17	17	64 82

The large amount of nitrate is of course no evidence that nitrification is stimulated, but is simply the result of the increased ammonia production, and accidental inoculation with nitrifying organisms.

§ 31. When the soil has been heated, however, it becomes unfitted for the development of the nitrifying organism. Apparently a toxic body is produced, which however only acts on the nitrifying organism and not on those producing ammonia. In one experiment soil was completely sterilised by heating to 130° for 45 minutes and then infected by admixture with a trace of ordinary soil; the production of ammonia and nitrate was as follows:

	At beginning	After 21 days	After 50 days
Nitrogen as nitrate	13·6	14·2	15·6
Nitrogen as ammonia	5·8	26·9	48·6
Total (parts per million of dry soil) ...	19·4	41·1	64·2

Pickering has already demonstrated the formation of a toxic substance by heat, and our results are in complete agreement with his on this point.

The toxic substance slowly disappears from the soil and ultimately nitrification once more becomes possible (cf. also § 45).

§ 32. *Denitrification.* Organisms decomposing or assimilating nitrates seem to be little influenced by toluene, but they are adversely affected, though not killed, by heat. The nitrate completely disappeared in 5 days from 50 c.c. of Giltay's culture solution inoculated with 5 grams of untreated or toluened soil and maintained at a temperature of 30°, but it persisted for 20 or 30 days when inoculated with heated soil.

§ 33. *Organisms suppressed by partial sterilisation.* Even a cursory examination of the soil reveals the fact that the bacterial flora has altered. Neither the heated nor the toluened soils possess the characteristic soil odour: the heated frequently smells somewhat musty and the toluened has a faint but quite distinct odour. The toluened soil often shows white spots like mould, which proved to be white streptothrix.

§ 34. Gelatine plate cultures were made by Koch's method of untreated and partially sterilised soils immediately after partial sterilisation, and again on the ninth day after moistening. There had been the usual enormous increase in number in the "toluene evaporated" and, to a less extent, the heated soil: this is recorded in Table 12. The organisms present on the various plates, and the proportions their colonies form to the whole assemblage, are given in Table 12.

In the untreated soil the white streptothrix and 8—11 predominate at first, followed by brown streptothrix and the two organisms 15 and 18, then come a number of others: moulds, mycoides, zopfii, fluorescens, 13, 17, 18, etc., none of which formed 10 per cent. of the colonies on the plate. After the soils have been kept moist for nine days there is a slight rearrangement: 8—11 now predominate, then follow the brown streptothrix, then the white and 13, whilst the other organisms remained as before, so far as could be judged.

The order in the toluened soil is different. White and brown streptothrix and 8 to 11 suffer less than the others and predominate directly after toluening. Nine days afterwards white streptothrix has gone ahead very considerably and is the principal organism present whilst the brown streptothrix formed less than 20 per cent. of the colonies. After a long period the brown streptothrix was much further diminished and the chief organisms were 8 to 11, 7 and white streptothrix. The difference in appearance of the plates is very striking; the colonies from the untreated soil look mainly brown, whilst those from the toluened soils are mainly white. It is curious that brown streptothrix

predominates over the white in the untreated soil, but not in the toluened soil. Where toluene is left in, however, the white streptothrix slowly suffers. Only three of the bacteria observed are killed by the short action of toluene—*B. fluorescens*, 17 and 18.

The effect of heat is much more drastic. Streptothrix, moulds, *B. mycoides, fluorescens, zopfii* and others are killed, leaving as survivors only 8 to 11, 13, 12, 16, 7, of which 8 to 11 and 13 much outnumber the rest. The flora of the heated soils is thus fairly simple.

Table 12. *Relative proportions of colonies on the gelatine plates.* (1) *Immediately after partial sterilisation*.*

	Untreated soil	Toluene evaporated	Toluene left in	Heated soil
Total number of organisms per gram	6,693,000	2,600,000	2,311,000	393
Percentage of colonies				
Above 30	White streptothrix 8—11		White streptothrix	8—11 13
20—30	Brown streptothrix	White streptothrix	8—11	
10—20	18 15	Brown streptothrix 8—11 7 13	14	
Below 10	Moulds *B. mycoides* *B. zopfii* *B. fluorescens* 7 12 and others	Moulds 26 *B. mycoides* *B. zopfii* and others	Brown streptothrix 13 *B. mycoides* *B. zopfii* and others	12 16 7
Absent		*B. fluorescens* 17 18	*B. fluorescens* 17 18 Moulds	*B. fluorescens* 17 18 Moulds Brown streptothrix White streptothrix *B. mycoides* *B. zopfii* 14 15 19 20—26

* Pending complete identifications some of the organisms are provisionally designated by numbers.

After nine days the proportions have somewhat changed:—

	Untreated soil	Toluene evaporated	Toluene left in	Heated soil
Total number of organisms per gram	9,814,000	40,620,000	2,617,000	6,294,000*
Percentage of colonies				
Above 30	8—11	White streptothrix	8—11	13
				8—11
20—30	Brown streptothrix			
	White streptothrix			
10—20	13	8—11	White streptothrix	
		Brown streptothrix		
		7		
Below 10	Moulds	*B. mycoides*	Brown	7
	7	Moulds	streptothrix	12
	15	*B. zopfii*	13	16
	14	14	18	
	18	15		
	B. mycoides	16		
	B. zopfii	13		
	B. fluorescens	18		

* After 4 days; 9 days' count lost by plates liquefying.

§ 35. The differences shown by the flora of the toluened and heated soils are much more marked than between the toluened and untreated soils and cannot in any case be correlated with the ammonia production curves. Attention has also been directed to the apparent loss of activity after toluening of the separate species examined (§ 26). We must therefore conclude that the change in type is less significant than the enormous increase in numbers of the decomposing organisms.

§ 36. It does not appear that any bacterial factor causes the comparative infertility of the untreated soil. Addition of the untreated soil extract to toluened soil caused no depression in the production of ammonia, or the number of organisms, but on the contrary a considerable increase; the organisms contained in the extract have no inhibiting effect, but multiply side by side with those present in the toluened soil. The extract was prepared by shaking 20 grams of soil with 100 c.c. of water and filtering through cotton-wool: nor did inoculation with *B. fluorescens,* the most striking organism suppressed by toluene, have any inhibiting effect. On the other hand addition of 5 per cent. of untreated soil, although at first without apparent action, after a time stopped the further increase in bacterial numbers and in ammonia. The results are set out in Table 13 and Curve 3 (p. 140).

Table 13. *Number of organisms per gram of soil.*

Treatment of soil	After 20 days	After 38 days	After 61 days
Not infected:			
Untreated soil	6,000,000	7,500,000	9,500,000
Toluene evaporated	28,000,000	31,800,000	60,100,000
,, and sterilised soil extract	32,000,000	31,600,000	67,000,000
Toluened and infected with soil extract	61,300,000	45,200,000	166,600,000
5 per cent. untreated soil	32,000,000	46,900,000	48,000,000
B. fluorescens	73,300,000	46,700,000	67,000,000
B. 9—11	33,600,000	30,400,000	104,000,000

Change in ammonia and nitrate.

Treatment of soil	Nitrogen as ammonia			Gain in nitrogen as nitrate	Total gain in nitrogen as ammonia and nitrate during 57 days
	At beginning	After 57 days	Gain		
Not infected:					
Untreated soil	2·3	4·8	2·5	0	2·5
Toluene evaporated	4·8	30·2	25·4	−1·1	24·3
,, and sterilised soil extract	7·6	33·9	26·3	−2·9	23·4
Toluened and infected with soil extract	7·1	44·9	37·8	+5·9	43·7
5 per cent. untreated soil	7·3	2·9	−4·4	+24·7	20·3
B. fluorescens	6·7	31·5	24·8	−5·5	19·3
B. 9—11	7·7	32·6	24·9	−6·7	18·2

§ 37. There is clearly some factor in the untreated soils which limits bacterial activity and which is put out of action by heating or by treatment with toluene. Other experiments lead to the same conclusion.

Arguments have been adduced in § 8, and need not be here repeated, against the view that the limiting factor is a toxin.

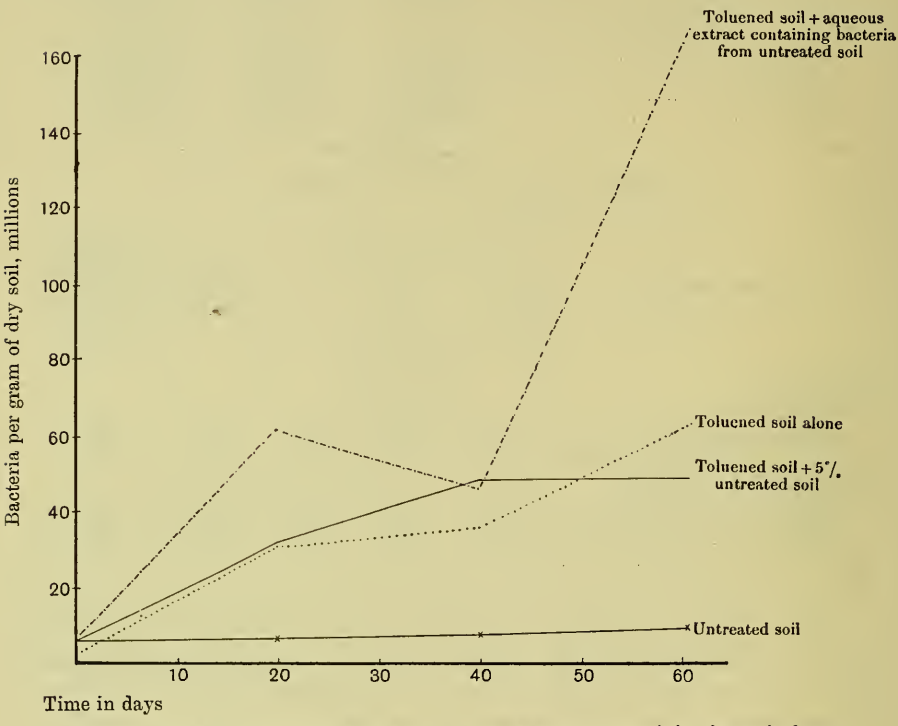
Ammonia produced from urea (as mgms. of nitrogen).

Treatment	16 hours	24 hours	37 hours	50 hours
Extract of untreated soil (5 c.c.) and toluened soil (25 gms.)	2·2	7·4	51·0	59·5
,, toluened ,, ,, ,,	2·2	8·7	52·4	61·0

§ 38. The limiting factor is probably biological since it takes time to operate when it is re-introduced into a toluened soil (§ 36). It occurs to a less extent in the *extract*: thus when an extract of untreated soil

is poured on to toluened soil, the decomposition of urea is only slightly less than when an extract of toluened soil is added.

However, when we applied a more sensitive test and mixed the extracts of untreated soil and of toluened soil in equal proportions we found that the limiting factor is also present in the extract.



Curve 3. Effect of untreated soil, and of aqueous extract containing bacteria from untreated soil, on the bacterial activity in the toluened soil (Table 13).

§ 39. These facts point to large organisms as the limiting factor. Examination was made for algae and for protozoa by the following methods:

(1) *Algae.* A solution containing per litre 2 gms. sodium nitrate, 0·5 gm. each monopotassium, phosphate, sodium chloride, magnesium and calcium sulphates was sterilised and inoculated with 5 gms. soil per 100 c.c. solution, calcium carbonate was also added. The flasks were kept in a warm place exposed to light, and after a few weeks a vigorous algae growth had developed from the untreated soil, only very little from the toluened, and none from the heated soil. Partial sterilisation has therefore removed algae.

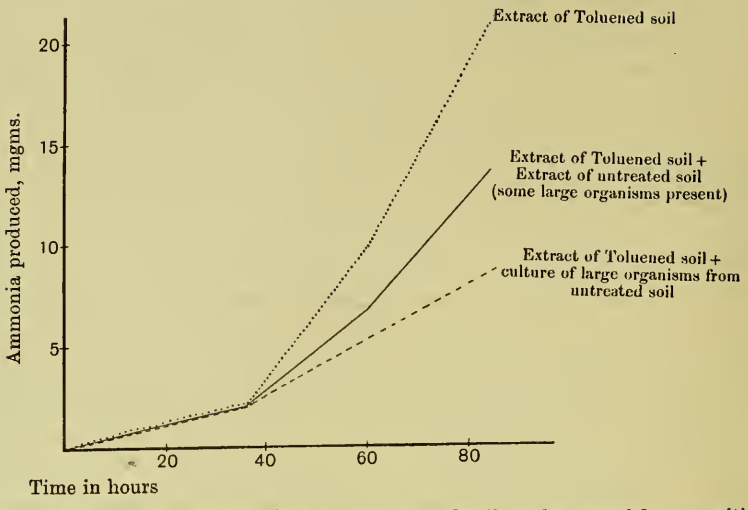
(2) *Protozoa.* Soil was inoculated into a sterilised 2 per cent. infusion of hay, or, in other experiments, into a sterilised mixture of 2 per cent. hay infusion and 1·5 per cent. agar which was then poured into Petri dishes. After a time large organisms were picked off from the untreated soil cultures, including amoebae and ciliata. Some of these were kindly examined by Professor S. J. Hickson and found to be mainly *Colpoda cucullus.* The toluened soil cultures only contained very small ciliated infusoria, the heated soil cultures contained none. The extract of untreated soil generally contained small protozoa. From the fact that *Colpoda* is a common hay infusion form we may infer that it is widely distributed and capable of living and multiplying in the soil. Its main food seems to be bacteria, and its action must therefore be to keep down the number of bacteria and consequently the amount of decomposition they effect. We may therefore conclude that organisms of this class constitute a factor limiting bacterial activity and fertility in ordinary soil.

Even if certain protozoa and organisms like the algae have no direct effect on bacteria they must be severe competitors in the struggle for existence in so far as they are actually living in the soil. The effect of this large organism is well shown in the following experiment on the rate of decomposition of peptone by the extract of toluened soil. Addition of an equal volume of the extract of untreated soil reduced the rate of decomposition considerably and addition of a mixed culture of the large organisms obtained from untreated soil brought it down still more. The sterilised extract of untreated soil had no effect.

Table 14. *Effect of large organisms on the rate of decomposition of peptone by soil bacteria.*

	Ammonia in mgms. produced after		
	36 hours	60 hours	84 hours
Extract of toluened soil	2·1	9·8	20·9
Extract of toluened soil + large organisms from untreated soil	2·1	5·4	8·5
Extract of toluened soil + extract of untreated soil, containing large organisms	1·6	6·9	13·5
Extract of toluened soil + above extract sterilised	2·0	9·8	20·8
Extract of untreated soil alone	1·9	6·6	12·0

The results are plotted on Curve 4.



Curve 4. Effect of large organisms from untreated soil on the rate of decomposition of peptone by soil bacteria (Table 14).

§ 40. Not only does partial sterilisation kill these destructive and competing organisms and thus make the conditions more favourable for the new bacterial flora, but it probably also increases the food supply. We have been able to observe under the microscope a dissolution of the killed protozoa by the bacteria. It is not possible as yet to form any estimate of the amount of nitrogen thus supplied as food, but it cannot be anything like the amount of ammonia ultimately produced in the soil.

§ 41. As already remarked, toluene does not kill all the large organisms but leaves at least one which in course of time developes. It is probable that this organism is concerned in the falling off in activity of the bacterial soil after a long period as indicated by the second crop (Table 1), the drop in the rate of oxidation (§ 20) and the fall in bacterial numbers.

§ 42. While the evidence must be regarded as fairly complete that the removal of large unfavourable organisms is one cause of the improvement effected by partial sterilisation, we by no means wish to imply that it is the only one. It is quite possible that there are other factors involved. We found, for instance, a nitrogenous substance in the soil which was very soluble in toluene, the distribution of which would no doubt be affected by toluening. Some of the catalytic changes brought about by soil, *e.g.* the decomposition of

hydrogen peroxide, seemed to be influenced by partial sterilisation. Heat certainly causes decomposition and increases the food material available. These and other factors are under investigation.

§ 43. *Plant growth in partially sterilised soils.* So far as the plant is concerned the difference between the partially sterilised and the untreated soils may be briefly summed up. In the partially sterilised soil organic matter decomposes more rapidly with the production of a greater amount of ammonia, but no nitrate. Plants make greater growth and contain an increased percentage of nitrogen and of phosphoric acid.

In the earlier paper on partial sterilisation the question was raised: In what form do plants take up their nitrogen from partially sterilised soils? The pot experiments indicate that it cannot be taken up as nitrate, but they are not conclusive by reason of the liability to re-infection. In order to make the evidence quite clear a number of plants were grown in conditions where infection did not take place.

The soil was filled with all proper precautions into sterilised Woolff's bottles with three necks. Through the centre neck the sterilised seed was dropped and a plug of cotton-wool inserted; in each of the others was fixed a glass tube, one for the water supply reaching to the bottom of the bottle, the other, for the air supply, just dipped inside and was plugged with cotton-wool. The soil was weighed, and the nitrate was determined; the quantity of nitrate present in each bottle was therefore known. The plants were kept in a special glass house kept as free as possible from dust.

Water was added at regular intervals so that 18 per cent. should always be present; the necessary amount was ascertained by weighing the whole apparatus on each occasion. The difficulty of adding water without at the same time introducing bacteria was overcome by permanently connecting a Pasteur flask filled with sterilised water to the Woolff's bottle, and transferring water from the flask to the soil in the ordinary way. When the crop was harvested at the conclusion of the experiment examination was made for the nitrifying organism which, however, was found to be absent. The soils were then again partially sterilised and sown with a second crop; the results are given in Table 15, and photographs of typical plants in Plate VIII, Fig. 3.

In a second series of experiments nitrifying organisms were added.

Six bottles formed the unit in each experiment.

§ 44. It is quite clear that the plants have got their nitrogen from some source other than nitrates. The percentage of nitrogen in the dry matter of the rye is at its lowest (= 2·07 per cent.) in all cases

TABLE 15.

Series 1. Crops grown without addition of nitrifying organisms.

	1st crop. Rye			2nd crop. Wheat		
	Dry matter produced, grams	Nitrogen in dry matter, per cent.	Nitrogen taken from soil, grams	Dry matter produced, grams	Nitrogen in dry matter, per cent.	Nitrogen taken from soil, grams
Untreated soil...	·836	2·07	·0173	·204	1·612	·0032
Toluened soil ...	1·022	2·41	·0246	·885	1·096	·0097
Heated soil	·994	3·34	·0332	·979	1·900	·0186

Series 2. Crops grown with addition of nitrifying organisms.

Untreated soil...	·536	2·11	·0113	·353	1·797	·0063
Toluened soil ...	1·159	1·99	·0231	1·093	1·115	·0122
Heated soil	1·024	3·18	·0326	1·321	2·029	·0268

Total nitrogen taken by crop, compared with nitrogen originally present as nitrate.

	Nitrogen in 1st crop, grams	Nitrogen in 2nd crop, grams	Total in 1st and 2nd crops	Nitrogen as nitrate in soil	Difference, being nitrogen assimilated otherwise than as nitrate
Toluened soil (*Series* 1)...	·0246	·0097	·0343	·0081	·0262
Heated soil (*Series* 1).....	·0332	·0186	·0518	·0081	·0437

where nitrate is being produced in the soil and presumably forms the chief nitrogenous food of the plant, *i.e.* in the untreated soil, and the untreated and toluened soils inoculated with the nitrifying organism. It is higher where no nitrate is being formed, *i.e.* in the two heated soils and the uninoculated toluened soil. The introduction of the nitrifying organism into the heated soil has had little or no effect in reducing the percentage of nitrogen in the dry matter of the plants. Wheat behaves differently, and requires further investigation.

Nitrification is therefore not essential to plants, but it may be economical. A greater weight of dry matter is formed for each unit of nitrogen assimilated as nitrate than as other compounds.

§ 45. Whilst the first crop was growing the nitrifying organisms inoculated into the heated failed to develope, being inhibited, probably, by a toxic body (§ 31). But during the time the second crop was growing the added organisms developed abundantly, a result suggesting that the toxic body slowly disappears from the soil.

a *b* *c*

Fig. 1. 1st crop, Rye.

a *b* *c*

Fig. 2. 4th crop, Wheat.

a. Heated soil. *b*. Untreated soil. *c*. Toluened soil.

Crops grown on partially sterilised soils. After partial sterilisation three crops were grown—Rye, Buckwheat, Spinach. The soil treatment was then repeated, and a fourth crop, wheat, taken. No manure was added.

Fig. 3. Wheat growing under aseptic conditions.

1. In untreated soil.
2. In toluened soil.
3. In heated soil.

Gelatine cultures in Petri Dishes. $\frac{1}{1000000}$th of a gramme of the respective soils was used for inoculation.

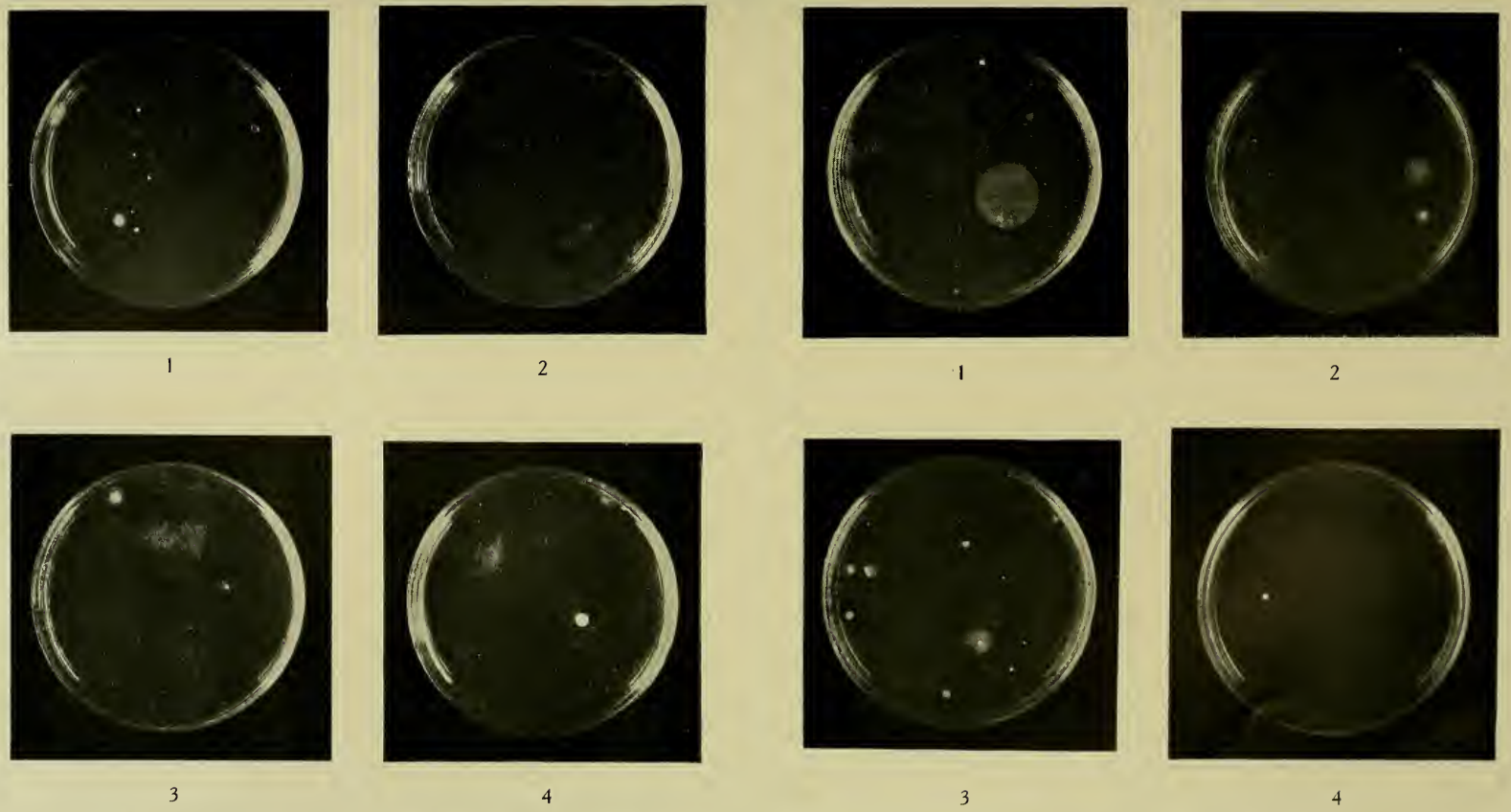


Fig. 4. Cultures from soils immediately after treatment.

1. Untreated soil (8 colonies).
2. Heated soil (0 colonies).
3. Toluened soil (toluene evaporated) (2 colonies).
4. Toluened soil (toluene left in) (4 colonies).

Fig. 5. Cultures from soils, 10 days after treatment.

1. Untreated soil (10 colonies).
2. Heated soil (30 colonies).
3. Toluened soil (toluene evaporated) (19 colonies).
4. Toluened soil (toluene left in) (2 colonies).

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ESTIMATION OF CALCIUM CARBONATE IN SOILS.

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This work was undertaken at the suggestion of Mr A. D. Hall, whose attention was drawn to the subject by some abnormal results obtained in the estimation of calcium carbonate in certain soils from different parts of the world characterised by their high humus content and their acid reaction to litmus paper. Boiled with diluted sulphuric acid (1 : 1 H_2SO_4), most of these soils yielded an amount of carbon dioxide (estimated by Brown and Escombe's double titration method) equivalent to a percentage of 1—3 of calcium carbonate in the air dried soil: while others yielded still higher amounts. It is quite possible that a soil may be acid in reaction and yet contain carbonate[1], but such percentages are quite incompatible with the strong acidity present in these cases. It seemed possible that the carbon dioxide evolved from such soils when boiled with acid resulted from the decomposition of unstable organic matter: and this is the conclusion arrived at by the writer.

The apparatus used in the investigation was that described by Amos[2].

Two soils which showed specially high percentages of carbonate (as calculated from the carbon dioxide evolved) were selected as test soils. These will be referred to under their Laboratory numbers, Ohio I, and Transvaal III: in addition use was made of many other soils both acid and normal. They were used in an air dried condition, and powdered till they would pass through a sieve with square holes, passing particles less than 0·4 mm. in diameter. The test for decomposition of organic matter with production of carbon dioxide was carried out as follows. 10 grams of soil were placed in a basin with 50 c.c. of boiled water, and 15 c.c. of strong hydrochloric acid added. The basin was placed in a

[1] Hall, Miller, and Gimingham, *Proc. Roy. Soc.* B. 1908, 80, 196.
[2] *Journal of Agricultural Science*, 1905, 1. 322.

desiccator over strong caustic soda and a good vacuum obtained by means of a Fleuss pump. The whole was allowed to stand for several hours in order to ensure the decomposition of the carbonate, after which time the contents of the basin were washed into the distilling flask of the carbon dioxide apparatus with 50 c.c. of water, and boiled for twenty minutes. The absorbing Reiset tower was then detached and the carbon dioxide estimated. The distillation was then continued for a second period of twenty minutes, and also for a third, with the following result. The figures are given in milligrams of carbon dioxide per 100 grams of soil.

Soil	1st 20 mins.	2nd 20 mins.	3rd 20 mins.
Transvaal III	422	224	211
Ohio I	316	171	136

The results indicate the continued decomposition of something in the soil which yields carbon dioxide but which can hardly be calcium or any other earthy carbonate. Even if any carbon dioxide remained dissolved in the acid solution standing in the vacuum, it would have been removed during the first boiling, so that the carbon dioxide obtained in the second and third boilings must have been freshly formed by the slow decomposition of the organic matter in the soil.

An attempt was then made to minimise the decomposition of organic matter by substituting ammonium chloride for the acid—

$$CaCO_3 + 2NH_4.Cl = CaCl_2 + (NH_4)_2CO_3.$$

Hartleb and Stutzer[1] used ammonium chloride instead of hydrochloric acid, and estimated carbonate as ammonia. This method is open to criticism, and was found to be quite unreliable for acid soils, the free acid of which combines with the ammonia produced and thus renders the results too low. In the case of a New Zealand acid soil less ammonia came over in the distillation of ammonium chloride with soil than in the blank distillation of the ammonium chloride solution itself.

There can, however, be no objection to a distillation with ammonium chloride if the carbon dioxide arising from the dissociation of the ammonium carbonate is estimated, and the carbonate calculated from this figure: this was done by the writer. 10—20 grams of the fine soil were put into the distilling flask along with 75 c.c. of boiled water.

[1] *Zeit. angew. Chem.* 1899, xii. 448.

50 c.c. of a 20 % solution of ammonium chloride were introduced by means of a 3-way funnel, and the distillation was continued for thirty minutes after the contents of the distilling flask reached the boil. The same apparatus as before was used, with the addition of an acid trap containing dilute sulphuric acid and fitted with a condenser. This trap was provided to prevent ammonia from reaching the absorbing Reiset tower, as it was found that ammonia interfered with the phenol-phthalein titration rendering it slower and less sharp. The results obtained by this method (expressed as before in milligrams of carbon dioxide per 100 grams of air-dried soil) were always lower than those obtained with hydrochloric acid.

Soil	1st 30 mins.	2nd 30 mins.
Transvaal III	83	65
Ohio I	140	79

A series of soils yielded on the average 52 milligrams more carbon dioxide per 100 grams of soil by distillation with hydrochloric acid than with ammonium chloride. The subsoils agreed very closely, a difference of only 12 milligrams carbon dioxide per 100 grams soil being obtained on the average. This points to the organic matter, which is comparatively speaking absent in the subsoil, as the source of the extra carbon dioxide evolved from the surface soil.

The next step was to ascertain whether by boiling such soils with water alone any evolution of carbon dioxide took place. This was invariably found to be the case. The results, calculated as before, are given in the following table. 125 c.c. of water was used and the boiling continued for 30 minutes.

Soil	1st 30 mins.	2nd 30 mins.
Transvaal III	66	—
Ohio I	94	39
New Zealand Virgin Pasture...	47	—
Plot 11 Rothamsted Pasture...	22	—

As it was highly improbable that these soils, all of which showed a strong acid reaction to litmus, contained any appreciable amount of carbonate, and as they gave off carbon dioxide on boiling with water

alone, it seemed impossible in such cases to obtain an accurate estimation of the carbonate by any method in which the soil was subjected to the decomposing effect of water boiling under atmospheric pressure.

Extraction with ammonium sulphate in the cold was next tried. The soil was shaken for twelve or more hours with a strong solution of ammonium sulphate, and allowed to stand till the supernatant liquid was quite clear. An aliquot portion was then pipetted off by means of a filter pump (to avoid disturbing the fine sediment at the bottom of the extraction flask), and the carbon dioxide estimated by boiling in Amos' apparatus, a little sulphuric acid being added to prevent ammonia from reaching the absorbing Reiset tower. While negative results were got for carbonate in the acid soils tested, the normal soils always showed carbonate though in quantities below those estimated by direct treatment with acid. The carbonate could always be determined with a considerable degree of accuracy by the following procedure. First of all, the carbon dioxide was estimated by Amos' method. The same amount of soil was then boiled with dilute hydrochloric acid for a similar period of time under like conditions after standing in a vacuum as described in the first experiment to verify the decomposition of organic matter. The figure for the carbon dioxide *evolved from carbonate* was found by subtracting the amount of carbon dioxide evolved in the latter estimation from the total found in the former. The method is not free from objection owing to the difficulty of maintaining the experimental conditions exactly similar, but can be relied on as giving very satisfactory results. In normal alkaline soils containing 1—2 % carbonate of lime, the amount of carbon dioxide evolved on boiling with pure water was on the average 44 milligrams of carbon dioxide per 100 grams soil which corresponds to 0·1 % carbonate of lime. As the ammonium chloride method gave results that were much too low in comparison with those obtained in the manner described, it was abandoned as unreliable.

Extraction with water supersaturated with carbon dioxide also failed to give satisfactory results. The excess of carbon dioxide was boiled off and acid added to decompose the precipitated carbonate, but the results obtained were very erratic.

Finally, a distillation with very dilute acid at reduced pressures was tried and adopted as giving results which were very satisfactory compared with those obtained by distilling the soil under atmospheric pressure. Transvaal III, which, as the ammonium sulphate extraction showed, contained no carbonate, yielded when boiled with water alone

under reduced pressure 7 milligrams of carbon dioxide per 100 gm., an amount which scarcely exceeds the unavoidable experimental error, and certainly shows that water alone did not decompose any appreciable amount of organic matter under these conditions. 20 grams of the Transvaal soil were now taken and boiled for 20 minutes at 50° C. with 2 c.c. strong hydrochloric acid and 100 c.c. water. 19 milligrams of carbon dioxide per 100 grams soil were obtained and on continuing the process 11 milligrams. The contents of the distilling flask were now boiled for 20 minutes at atmospheric pressure and 158 milligrams were now evolved. It will be observed that the strength of the acid is an important factor in determining the amount of decomposition, as this soil yielded 422 milligrams carbon dioxide when boiled with the stronger acid used in the test for the decomposition of organic matter. The Sprengel water pump was used to reduce the pressure, and considerable care must be exercised during the experiment, especially when allowing air to pass through the apparatus on the completion of the decomposition of the carbonate.

The results obtained by this method with eight acid soils tested never, with the exception of Ohio I and Transvaal III, rose above 9 milligrams carbon dioxide per 100 grams soil, while on boiling at atmospheric pressure ten times as much was found, and that after all carbonate must have been decomposed. 9 milligrams of carbon dioxide corresponds to 0·02 % calcium carbonate, and whether a soil contains this amount or no carbonate at all is a matter of no great importance.

The following table gives a comparison of the results obtained for the carbon dioxide in Transvaal III and Ohio I by the various methods tried.

Soil	With 1:1 H_2SO_4	With dilute HCl at atmospheric pressure	With $NH_4.Cl$ (distillation)	With boiling water	With $NH_4.Cl$ (extraction)	With dilute HCl under reduced pressure
Transvaal III.	1540	422	83	66	0	19
Ohio I	2772	316	140	94	0	12

An attempt was made to isolate from Transvaal III and Ohio I a portion of the organic matter which, boiled at atmospheric pressure with dilute hydrochloric acid, should give off a much larger percentage of carbon dioxide than the soil itself. For this purpose part of the

humus was extracted with 4 % ammonia after preliminary treatment with 1 % hydrochloric acid, which was removed before extracting with ammonia. Curiously enough, the percentage of carbon dioxide evolved from the humus, which was dried in a desiccator over sulphuric acid, did not increase, although the same experimental conditions were maintained as before.

Amos' observations on the occlusion of carbon dioxide in soil were repeated and confirmed. It was found that occlusion of carbon dioxide in air-dried soil does not take place to any appreciable extent.

I have to thank Dr N. H. J. Miller of this laboratory for his continued advice and assistance during the progress of this work.

Summary and Conclusions.

Boiling acid at atmospheric pressure decomposes organic matter in soil with evolution of carbon dioxide, and thus renders the results obtained for carbonate too high. Where there is a fairly large percentage of carbonate, the error introduced in this way is of no great importance, but in soils containing less than 1 % of calcium carbonate and especially in acid soils, the error introduced by thus boiling with acid may be very considerable.

The weaker the acid used the better so long as there is fair excess. The writer recommends for acid soils and those containing low percentages of carbonate (as can be seen by making a rough preliminary test), 2 c.c. of strong hydrochloric acid and about 100 c.c. of water: 20 grams of soil should be used when the amount of carbonate is small. The acid may be conveniently added by making up a solution containing 100 c.c. of strong hydrochloric acid per litre, and introducing 20 c.c. of this solution along with 80 c.c. of water. For most soils, 5 c.c. of strong hydrochloric acid to 100 c.c. of water will be found convenient.

If possible distillation under reduced pressure should be used, as under this condition practically no decomposition of organic matter takes place, while carbonate is readily decomposed: the distillation should be continued for twenty minutes at a temperature of about 50° C.

Since the above paper was ready for publication we have learnt of the death of the author at Breslau on May 13th. After working for a year in the Rothamsted Laboratory Mr Marr proceeded to Breslau to work under Dr Th. Pfeiffer, and there the course of a promising worker, who endeared himself to all with whom he came in contact, was untimely cut short. A. D. H.

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THE AMOUNT OF FREE LIME AND THE COMPOSITION OF THE SOLUBLE PHOSPHATES IN BASIC SLAG.

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Basic Slag owes its value as a source of phosphoric acid to the fact that it is essentially basic in its character, and can be used on land where an acid manure of the character of superphosphate is not to be recommended.

As no figures were available on the subject it seemed interesting to determine how much of the lime which it contains existed in the free uncombined condition. It has been stated that in some cases this is as much as 20 %.

With a view to this determination four samples of freshly ground slag were obtained direct from the makers through the kindness of the Lawes Chemical Manure Company.

An attempt was made to follow the method of Stone and Scheuch[1] for the estimation of lime in commercial quicklime. The method consists in shaking a weighed quantity of the slag with a 10 % solution of cane sugar, filtering and titrating the lime with standard acid. However it was found that in the case of some of the slags this solution was darkly coloured and quite impossible to titrate, and contained in addition to the lime considerable quantities of iron. Further on acidifying the solution there was a considerable evolution of hydrogen sulphide. It was found that calcium sulphide dissolves to some extent in the sugar solution, 100 c.c. dissolving ·0174 gram of calcium.

This method was then abandoned—as were also others depending on the reaction of ammonium and sodium carbonates with the lime present. In all of these the reaction was interfered with by the sulphides present, and by the fact that some phosphoric acid compound was also attacked.

[1] *J. Amer. Ch. Soc.* 1894, xvi. 721.

The principle of the method finally adopted is to shake the slag for a considerable time with carbon-dioxide-free distilled water, and titrate with standard acid, using phenol-phthalein as an indicator.

The details of manipulation are as follows. A quantity of slag varying from 1 to 2 grams is shaken for 24 hours in an end over end shaker with 300 c.c. of water freed from carbon dioxide. The whole is then poured into a large Buchner funnel and filtered with pressure. The time taken for filtration is very small, so that the amount of hydrate changed into carbonate cannot be large. The slag is washed back into the flask and the process repeated. In the author's determinations the extractions were continued until the amount dissolved fell below ·0008 gram CaO.

The method probably gives results that are somewhat too low, owing to the conversion of a small amount of the hydrate into carbonate during the process of filtration, although this is probably compensated for to some extent by the fact that other calcium compounds in the slag are also to a small extent attacked, as it seemed impossible by continued extraction to obtain a solution which was not slightly alkaline to phenol-phthalein.

The point which the author adopted as the limit was usually reached at the third extraction.

Four determinations of lime in the same slag gave the following results:

5·05
5·24
5·22
5·99 } per cent. free lime.

It was considered that the results were close enough to make the method a useful one.

In the four samples of slag considered the percentages of CaO were as follows:

A. 4·69
B. 5·29
C. 1·28
D. 5·37

The numbers being rather lower than was expected it was suggested that this might be owing to the conversion of some of the oxide into carbonate as the result of storage.

Determinations were made of the carbonate present by the method

suggested by A. Amos[1]. A tube containing silver sulphate was inserted before the absorption Reiset, to prevent the hydrogen sulphide given off interfering with the result.

The figures for the percentage of calcium carbonate in the four slags are:

A. 2·08
B. 2·14
C. ·72
D. ·43

Thus in the four slags examined, which are believed to be typical ones, the percentage of lime present both as carbonate and oxide does not exceed $7\frac{1}{2}$ %.

Table I.

SLAG	1st	2nd	3rd	4th	5th	Total CO_2 sol.	Total in slag	CO_2 sol. % of total
A.	5·349	3·380	1·405	·454	·244	10·832	15·81	66·99
	5·620	3·321	1·382	·532	·244*	11·099		
Mean...	5·484	3·350	1·393	·493	·244	10·965		
B.	6·080	4·310	1·880	·559	·259	13·088	18·61	63·57
	6·324	4·282	1·860	·7093	·259*	13·261		
Mean...	6·202	4·296	1·87	·634	·259	13·179		
C.	7·811	3·471	·804	·210	·110	12·307	18·62	65·20
	7·166	3·966	·725	·457	·153	12·487		
	7·120	3·546	1·067	·300	·110	12·956		
Mean...	7·365	3·661	·855	·322	·124	12·584		
D.	5·750	5·350	2·630	·655	·243	14·628	22·30	65·60
	5·450	5·330	3·870	·490	·216	14·956		
Mean...	5·600	5·340	2·750	·572	·229	14·792		

It seemed to be a point of interest to determine what influence this amount of free lime had on the action of the solvents employed for determining the soluble phosphoric acid, and whether it was correlated in any way with the amount of the latter. A solution of carbon dioxide was the first solvent employed, and five consecutive extractions were made with this on each slag.

[1] *Journal Agric. Science*, Vol. I. part 3.

The solution was one as far as possible saturated at atmospheric pressure by diluting the solution obtained from a sparklet apparatus to double its volume and allowing it to stand in contact with the air for some time.

The determinations of phosphoric acid are given in Table I. The irregularities in the figures are doubtless due in great part to the difficulty in getting a solution of constant composition.

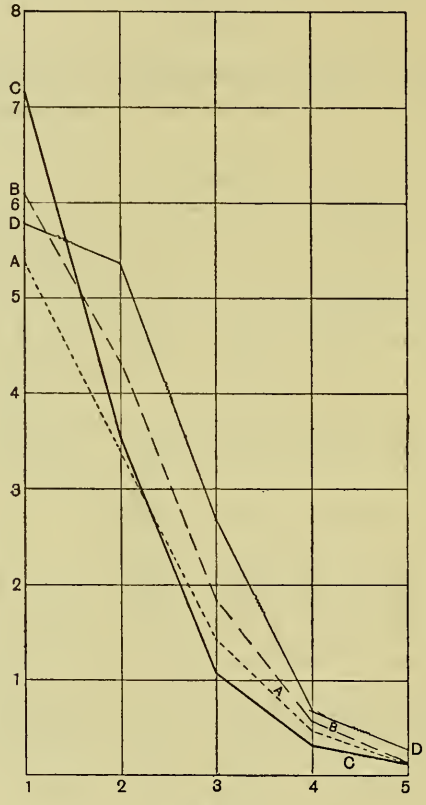

Fig. 1.

It will be seen that the quantity dissolved in no case amounts to 70 % of the total phosphoric acid present, and that the proportion is very much the same for all the slags. This fact would lead one to the conclusion that the easily soluble constituent, whatever it be, is the same in each case.

In Fig. 1 the above results are shown in a graphic form, the percentages of phosphoric acid being set out as ordinates, and the

number of extractions as abscissae. It would seem from these that had the extractions been pushed further more phosphoric acid might have been dissolved out. The difficulties of determining such small amounts of phosphoric acid made it impossible to do so. The curves are fairly regular except in the case of *B* and *D*, which show some disturbance, considerable in the case of *D*, at the beginning.

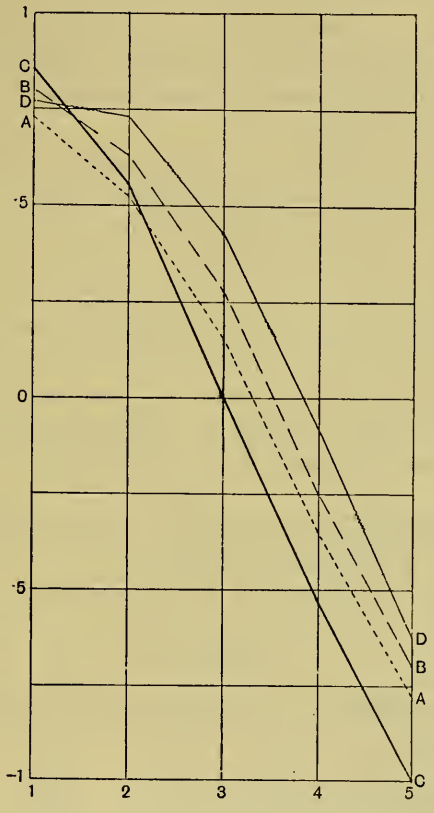

Fig. 2.

This is more clearly seen if, instead of the actual percentages obtained, the logarithms of these numbers are plotted, Fig. 2. The effect which is evident in the case of all four, but most marked in the case of *D* and *B*, is doubtless due to the presence of free lime. If the carbon dioxide were reacting with a single body these logarithmic curves should be straight. As it is it will be noticed that they become so after the first or at the furthest the second extraction, and further that those

containing the most lime show the greatest and that containing the least lime the least deflexion.

The first action of the carbon dioxide would almost certainly be the conversion of the free lime into carbonate. The mechanism of the following reactions comprising the conversion of carbonate into bicarbonate and the solutions of the phosphoric acid compounds is quite obscure. It would be reasonable however to expect, assuming the soluble phosphoric acid compound to be the same for the four slags, that the slag containing the least quantity of free lime should show the highest percentage of phosphoric acid soluble at the first extraction. This is precisely what occurs.

Thus owing to the small mass of the carbon dioxide entering into the reaction, the extent to which the phosphates are attacked is masked by the presence of varying quantities of free lime. Hence it follows that although the natural solvent in the soil is carbon dioxide, it is not possible in the case of basic slag to make use of it as a solvent for the determination of the soluble phosphoric acid.

It would seem probable, from the fact that the logarithmic curves approach to straight lines and that they run fairly parallel to each other, that the substance attacked is essentially the same in all of the slags. The probable composition of this compound will be discussed later.

Further determinations were made of the amount of phosphoric acid soluble in a 1 % solution of citric acid.

In this case three extractions were made. The results are given in Table II.

TABLE II.

Phosphoric Acid soluble in 1 % *Citric Acid. Shaken for* 24 *hours.*

Slag	1st extraction	2nd extraction	3rd extraction	Total sol.	Total sol. / Total	1st extraction / Total
A	13·84	1·232	·052	15·114	·9562	·8754
B	16·23	1·098	·045	17·373	·9331	·8723
C	14·06	·244	·030	14·334	·7695	·7550
D	20·88	·676	·015	21·571	·9672	·9366

Here in presence of a much larger mass of acid the small amount of lime has no longer any effect. Further, in 3 of the 4 slags as much as 93—97 % of the total present is dissolved. It is worthy of note that in the case of *C* and *D* the percentage of total phosphoric

acid, soluble in carbon dioxide solution, is the same, whereas in the case of the citric acid solution it is vastly different. This would suggest in the case of *D* the presence of compounds unattacked by the carbon dioxide.

It is a well-known fact that the effect of fine grinding, which will increase very much the surface in contact with the solvent, has a very large effect on the solubility of basic slag. That this has no effect on the very soluble phosphates is evident from the results with carbon dioxide, as *C* and *D* both show the same percentage of total phosphoric acid soluble, although the amount of *C* passing through a 0·2 mm. sieve is 76·60, while of *D* 98·21. The effect of the grinding is however shown in the citric acid solution. Thus it would appear that in basic slag there are at least two sources of phosphoric acid, one of which is very readily soluble in a weak acid like carbon dioxide, and one or more which are attacked by citric acid to an extent depending on the amount of surface exposed.

A portion of *B* was finely ground so that the whole passed through a 0·2 mm. sieve and citric acid extractions made as before.

		1st	2nd	3rd	Total	$\frac{1st}{Total}$
B.	Original sample	16·23	1·098	·045	18·61	·8723
	Finely ground sample...	17·28	1·022	·099	18·61	·9286

As regards the more difficultly soluble phosphate, as the solubility seems to depend so much on the surface of the slag, probably also the time during which the two are in contact is an important factor.

That this is so is seen in the case of *B*. Two series of determinations being made, one with 1 % citric acid shaken for 24 hours as above and another for ½ hour with 2 % citric acid as recommended under the regulations of the Board of Agriculture, the figures are given below:

		1st	2nd	Total	$\frac{\frac{1}{2}\text{ hour}}{24\text{ hours}}$	$\frac{1st\ \frac{1}{2}\text{ hour}}{1st\ 24\text{ hours}}$
A.	24 hours...	14·020	1·810	15·760		
	½ hour ...	13·13	1·808	14·938	·985	·941
B.	24 hours...	16·750	1·060	17·810		
	½ hour ...	14·460	2·440	16·900	·949	·692

It will be seen that the total dissolved may be regarded as the same, but considering the first extraction only there is a considerable difference and apparently a difference by no means the same for different samples of slag.

The question as to what is the soluble phosphatic compound in basic slag has been regarded as settled for a long time. It has always been

believed and taught that the body was a calcium phosphate of the composition $(CaO)_4P_2O_5$. In 1887, Stead and Ridsdale[1] described some large and apparently pure crystals of this composition that they obtained from basic slag.

This and a statement of Hilgenstock's seem to be the ground on which this belief has been based in spite of the fact that in Jan. 1895 Stead published another paper[2] in which the former paper is practically contradicted. As this latter work seems very generally to have been overlooked it may not be out of place to give at some length the conclusion arrived at.

In the first place the author states, "that of the phosphates contained in basic slag the most soluble consists of a chemical union of tetra-calcium phosphate and mono-calcium silicate. The more insoluble phosphates are in the form of hexagonal needles and flat plates and appear to consist essentially of tetra-calcium phosphate, which however varies in solubility in different specimens. Some varieties are as insoluble as coprolites and nearly as insoluble as apatite."

The above appears very much at variance with the usual opinion of the solubility of tetra-calcium phosphate.

What really is of still greater importance is the fact that in the large number of slags which Stead examined, "there was an entire absence of tetra-basic calcium phosphate crystals and a constant recurrence of blue crystals" the composition of which he states to be $(CaO)_4P_2O_5$, $CaO \cdot SiO_2$ containing

$$Ca\ 56{\cdot}578\ \%,\ SiO_2\ 10{\cdot}791\ \%,\ P_2O_5\ 29{\cdot}146\ \%.$$

Several attempts were made to obtain some crystalline specimens of slag. These however proved difficult to obtain, the makers stating that crystals were by no means common, and only occurred in certain balls of slag.

Finally, Messrs Albert were able to send a crystalline sample, in which however there was no sign of the presence of crystals of tetra-calcium phosphate, those present being apparently the blue crystals described by Stead, in a more or less pure condition. A sample obtained by the author in Berlin showed the same composition.

The pure blue crystals being very minute it was not easy to obtain sufficient for analysis. The time occupied in picking them out was

[1] *Trans. Chem. Soc.* 1887, 601.

[2] *Proceedings of the Cleveland Institute of Engineers.*

long, as each was examined under a lens and those showing any adherent impurity disregarded.

It was decided to determine only phosphoric acid, calcium, and silica. The result is given below:

Phosphoric acid	26·30
Calcium oxide	46·71
Silica	11·02

These figures being in the ratio of one molecule of phosphoric acid, one of silica, and between four and five of calcium oxide.

The results of other analyses of the crystals which were not so pure as the above are given below:

	I	II	III
CaO.....	38·90	44·20	37·91
P_2O_5	19·45	21·53	
SiO_2	10·06	10·94	9·36
FeO.....	17·03		

The crystals could not be obtained pure in sufficient quantity to make a complete analysis possible.

The points brought out by the above are two: 1st the large amount of iron the crystals contain, 2nd the constant molecular ratio of 1 : 5 between the calcium and the phosphoric acid.

These analyses would rather point to a body of the general form $(MO)_5M_1O \cdot SiO_2 \cdot P_2O_5$ where M is calcium more or less replaced by ferrous iron, and M_1 ferrous iron.

These crystals are very soluble. They dissolve readily in carbon dioxide solution, and of the total phosphoric acid present in this slag 93·2 % is soluble in a solvent containing ·1 % of citric acid, or one-twentieth of the concentration usually employed.

The percentage composition of such a body and the analytical figures obtained from the pure crystals are given below:

Calculated composition $(CaO)_5FeO \cdot P_2O_5SiO_2$		Found in Crystals
CaO	50·54	46·74
FeO	12·99	not determined
SiO_2	10·83	11·02
P_2O_5	25·63	26·30

It will be seen that the figures are fairly well in agreement. The material at the author's disposal was not sufficient to enable him to proceed further, therefore he merely wishes to suggest the possibility of some such constitution as that given above.

One fact has, however, in the author's opinion been fully established, by Stead's work and confirmed by the present analyses that it is not

tetra-calcium phosphate which supplies the soluble phosphoric acid in basic slag, but a body in which the molecular ratio of phosphoric acid to lime is 1 : 5.

Consideration of the amounts of phosphoric acid and lime dissolved by carbon dioxide solution affords striking confirmation of this as regards the whole mass of slag.

If the first three extractions are considered it may be assumed that all the readily soluble bodies have been attacked as well as all the free lime dissolved in the form of bicarbonate. The total lime dissolved also was determined.

Sum of 1st three extractions	CaO	P_2O_5
Slag D	33·48 %	13·69 %

If the 5·56 grams of free lime found by the water extraction method be subtracted there remain dissolved 27·92 % CaO compared with the phosphoric acid.

The molecular ratio of these is

$$\frac{27{\cdot}92}{56} : \frac{13{\cdot}69}{142}$$
$$\cdot 498 : \cdot 0964$$
$$5 : 1$$

Thus of the total lime present in the slag which was 38·62 %, 5·8 was as oxide or carbonate, 27·68 was combined in readily soluble form leaving 5·17 combined with the remainder of the phosphoric acid.

V. F. Kroll[1] in a preliminary note says that the principal constituent of basic slag is a compound hitherto unknown, consisting of a silico-phosphate of lime and ferrous iron, which would seem to agree with the results obtained in the present paper.

The absence of crystals of tetra-calcium phosphate, which were undoubtedly obtained from basic slag by earlier observers, and the low percentages of free lime now found to be present in the slag, may be correlated with the increased percentage of phosphoric acid in slags of modern manufacture, less lime being nowadays employed in the dephosphorisation process than formerly.

In conclusion the author wishes to thank the Lawes Agricultural Trust for the use of their Laboratory and to express his great indebtedness to Mr A. D. Hall, who suggested this investigation, and whose kind advice has been invaluable throughout.

[1] *Stahl und Eisen*, no. 19, May 6, 1908.

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DIRECT ASSIMILATION OF AMMONIUM SALTS BY PLANTS.

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It has recently been shown[1] that the soil of some of the Rothamsted Grass Plots which have received ammonium salts for many years in succession has become distinctly acid and that, consequently, nitrifying organisms have become greatly reduced in numbers. Nitrification is limited to portions of soil directly in contact with the few particles of calcium carbonate still remaining in the soil. It is evident therefore that more or less of the nitrogen assimilated by the grasses must be in a form, or in forms, other than nitrate—probably mainly as ammonium salt. In view of these results it seemed desirable to obtain additional evidence of direct assimilation of ammonium salts by plants.

The question possesses a further interest in the case of leguminous plants, since whilst non-leguminous crops (whether able to assimilate ammonia or not) undoubtedly take up, under normal conditions, most of their nitrogen in the form of nitrates, we have no knowledge of the form of nitrogen appropriated by leguminous plants from their root nodules.

In 1890, Loew[2] showed that platinum black in presence of alkali produces ammonium nitrite from nitrogen and water, and suggested that assimilation of free nitrogen is accomplished in a similar manner. The examination by one of us, in 1890, of numerous fresh nodules showed almost invariably an alkaline reaction, sometimes very marked. When this view, assigning an indirect role to the nodule organism—the production of suitable physical and chemical conditions for the union of nitrogen with the elements of water—was put forward, fixation of nitrogen apart from the nodules had not yet been observed. Recently Loew and Aso[3] have suggested that ammonium nitrite is the first

[1] *Proc. Roy. Soc.* 1908, B. **80**, 196.
[2] *Ber.* 1890, **23**, 1447.
[3] *Bull. Coll. Agric. Tokyo*, 1908, **7**, 567.

compound produced, and that the nitrous acid is immediately reduced to ammonia. An experiment we made with beans taken from a garden, showed the presence of ammonia both in the root and in the nodules. A few grams of fresh nodules, and about the same weight of the roots from which they were taken, were extracted with 75 per cent. alcohol and the extracts distilled under reduced pressure with magnesia. The amounts of nitrogen as ammonia were as follows:—

In Roots N. = 0·016 per cent.
In Nodules N. = 0·043 per cent.

If it should be shown that nodules generally contain more ammonia than the roots, and that ammonia is readily assimilated by leguminous plants, the results would lend some support to Loew's suggestion. In this connexion it may be mentioned that Frank (27) looked for nitrates in the nodules of peas grown in soil and failed to find any, whilst the roots showed a distinct nitrate reaction both above and below the point at which the nodules were attached. In the case of plants grown in sand free from nitrogen, no nitrates could be detected in any parts. Frank also detected the presence of asparagine in lupin and pea nodules as well as in the roots. Assuming the initial process in nitrogen fixation to be the production of an ammonium salt, it is probable that some of the ammonia would at once pass into the roots. It does not follow, however, that all the nitrogen derived from the nodules is taken up in the same form, and it seems equally possible that the asparagine found in the roots may have been partly produced in the roots themselves and partly obtained from the nodules.

Before describing the experiments on assimilation of ammonium salts it will be desirable, as the prevailing ideas on the subject are anything but clear, to show in some detail what has been already done. As, however, the number of papers on the subject is considerable, attention will be confined chiefly to the more recent experiments in which nitrification has been taken into account[1].

The first experiments in which precautions were taken to avoid the possibility of nitrification were made by Pitsch (21) at Wageningen. In these experiments, which were commenced in 1885 and continued every year until 1894, various plants were grown in humus sand contained in metal pots, holding about 30 kilos. The general method employed was first to sterilise the contents of the pots, covered with cotton wool, by

[1] The earlier experiments are summarised in S. W. Johnson's *How Crops Feed*, New York, and references are given at the end of this paper.

suspending in an oil bath heated at 160—180°. The soil was next extracted (in the pots) with water to remove nitrates, and again sterilised. Nitrogen, in the form of ammonium sulphate and sodium nitrate respectively, was added to the soil, sometimes both in larger and smaller amounts. Occasionally ammonium phosphate was also employed. Each series of experiments generally included pots which had been neither sterilised nor extracted, as well as sterilised and extracted soils without addition of nitrogen. During growth sterilised water was supplied to the soil from below. Some time (not immediately) after the conclusion of the experiments the soil was examined for nitrates and in every case nitric nitrogen was found to be absent. The results showed that whilst ammonium salts were directly assimilated, without previous nitrification, the yields obtained with nitrate were generally better, the advantage of nitrate over ammonium salts being particularly marked during the early stages of growth.

In an experiment with Oats in 1890, Pitsch found that all the soils, at the conclusion of the experiment, contained ammonia (N. = 0·0015 to 0·0058 per cent.), and that this nitrogen, added to the nitrogen in the plants, amounted to considerably more than was contained in the manures. It was found moreover that the nitrate plants contained more than twice as much nitrogen as was supplied in manure. So that these plants evidently drew on the soil nitrogen, probably, for the most part, in the form of ammonia, and partly as soluble humus[1], produced in the process of sterilisation.

In his last experiments, Pitsch shows that additions of sodium chloride to the pots manured with ammonium sulphate considerably increased the yield. It would seem to be possible that the relatively low yields obtained in most cases with ammonium salts may have been in part due to unfavourable conditions as regards the mineral constituents of the soil.

The methods employed by Pitsch seem to be as satisfactory as possible in experiments on so large a scale. It is evident that the soils were not only thoroughly sterilised, but that the condition of sterilisation was maintained. But although the results show that the different plants grew in absence of nitrates, they fail to show that the nitrogen assimilated was exclusively in the form of ammonia.

In 1887, Frank (22) grew beans and sunflowers in water-cultures containing nitrogen as ammonium salt and as nitrate. The solutions

[1] Compare H. W. Wiley, *Landw. Versuchs-Stat*, 1898, **49**, 193.

were not sterilised, and the only precaution to avoid nitrification was to add calcium in the form of chloride instead of as carbonate. The solutions were found, however, to be free from nitrates and to contain ammonia at the end of the experiment. The beans grew fairly well when supplied with an ammonium salt, and the stems were found to be free from nitrates.

Müntz, in 1889 (24) experimented with beans, kidney beans, maize, barley and hemp which were grown in soil which was first extracted and then heated at 100°. The seeds were sterilised by dipping for a moment into boiling water, and the pots were kept in cases ("véritables cages de Tyndall") provided with openings, covered with cloth, to render the air passing in free from germs. At the conclusion of the experiment the soils were found to be free from nitrates. The different plants assimilated 49 to 915 m.g. of nitrogen, probably in the form of ammonia. There is, however, no proof that nitrification had been entirely absent. If the ammonium salts had been only slowly, and perhaps locally, nitrified all traces of nitrates might have been removed by the plants. In Pitsch's experiments as already mentioned, the soils were left for some time after the plants were taken out before being examined for nitrates, so as to allow time for further nitrification in the event of nitrifying organisms being present.

Griffiths (25), almost at the same time as Müntz, grew beans in sterilised water-cultures, with ammonium sulphate as source of nitrogen. The seeds were sterilised by remaining half-an-hour in copper sulphate solution, and the jars containing the solutions were placed under large bell-jars the openings of which were closed with cotton wool. The plants grew remarkably well for four weeks, and reduced the amount of nitrogen in the solution from 0·05 to 0·027 per cent.; no nitrate could be detected.

The next experiments, by Bréal (28), were made with *Poa annua*. Tufts of the grass growing in soil were dug up, and the roots washed until free from soil and then placed in water. New roots were soon produced, whilst the original roots left off growing. After cutting off the old roots the plants were supplied with dilute solutions of ammonium sulphate. It was found that after 24 hours all the ammonia had been taken up. In these experiments sterilisation was unnecessary as the time was too short for nitrification to occur.

Kinoshita (29), and, subsequently Suzuki (30), grew seedlings of various plants for short periods in solutions of ammonium salts and sodium nitrate, in order to compare the amounts of asparagine pro-

duced. It was found that ammonium salts are rapidly converted into asparagine, whilst nitrates tended to accumulate, and, during the short time the experiment lasted, generally failed to increase the amount of asparagine. The production of asparagine is promoted by the presence of sugar, and in absence of sugar, or other suitable material, it was found that ammonia may accumulate in the plants and eventually cause injury.

In 1898, Mazé (32) grew maize in sterilised water-cultures containing ammonium sulphate and sodium nitrate respectively; calcium carbonate 0·2 per cent. was added. Two months afterwards the plants were taken up, and it was found that the ammonium sulphate solutions still contained ammonia, and that no nitrate was produced. The plants grew about equally well in the two solutions. In later experiments (33), culture solutions were employed containing both forms of nitrogen in different proportions. The results showed that when the relations of ammonium sulphate to sodium nitrate were 1 : 2 or 1 : 4 the whole of the ammonia was utilised whilst some nitrate remained in the solutions.

Kossowitsch (35) experimented with peas in sterilised sand-cultures. Calcium carbonate was present in addition to the usual minerals, and the ammonium salt was added gradually during growth. The results showed that ammonium sulphate and sodium nitrate were equally suitable as sources of nitrogen. The solutions and sand to which ammonium sulphate had been added were found at the end of the experiment to be free from nitrates and nitrifying organisms; in some cases, however, it was discovered that other micro-organisms were present, and in some moulds.

Gerlach and Vogel (37) found that maize plants, grown in sterilised soil manured with ammonium sulphate, contained more nitrogen (0·418 gram.) than similar plants grown in the same soil without nitrogen; the soils were found to be free from nitrates at the conclusion of the experiment.

Krüger (38) made a large number of experiments with various plants grown in a sterilised mixture of soil and sand. Sterilisation was effected by heating the pots in steam for one hour on 6 days; the seeds were sterilised with mercuric chloride. At the conclusion of the experiment, the soils were examined and those containing nitrate excluded. The conclusion is drawn that ammonium salts and nitrates are equally suitable for mustard, oats and barley; that ammonia is, if anything, better than nitrates for potatoes, whilst for mangolds nitrates are decidedly better than ammonium salts.

The last experiments to be described are those of Ehrenberg (39), who grew oats in sterilised soil, and in sterilised sand, employing seeds sterilised with mercuric chloride. Nitrogen was added in the forms of ammonium sulphate and sodium nitrate in sterilised solutions after the sand and soil had been sterilised. Calcium carbonate was present. The results of both series were negative as regards ammonium salts, the plants failing to grow, and the conclusion is drawn that nitrification is essential to the growth of higher plants, at any rate in the case of soils of slight absorptive power. When, however, the amounts of ammonium salts employed are considered in relation to the amount of water present, it will be seen that the injurious effects were probably due to too great concentration. The sand (5 kilos. per pot) contained 10 per cent. of water, or 500 c.c., and the amount of ammonium sulphate present was 1·4 gram or 2·8 grams per litre. In the soil (3·8 kilos.) the amount of water was 20 per cent., or 760 c.c., and this contained 1·8 gram of ammonium sulphate per litre. It has been shown by Mazé (*loc. cit.*), that even 1 per thousand of ammonium sulphate is very injurious[1], whilst in the experiment just described the amounts were nearly twice, or nearly three times, as high supposing the salt to be equally distributed (which was probably not the case), and a good deal higher, locally, if not evenly distributed. It is stated that on turning out the pots a distinct odour of ammonia was noticed.

The results of all the experiments described above may be summarised as follows. The results of Griffiths and Mazé seem to prove conclusively that beans and maize assimilate ammonium salts as readily as nitrates. The same may be said of Kossowitsch's experiments with peas, for although sterilisation was imperfectly maintained, nitrifying organisms were completely excluded. Bréal's results may also be considered to establish the utilisation of ammonia (by *Poa annua*). The results obtained by Pitsch, Müntz, Gerlach and Vogel, and Krüger indicate that the various plants employed are able to grow in absence of nitrate—not with absolute certainty as regards Müntz's experiments—but fail to prove that ammonia was the sole source of nitrogen.

Experimental.

Seed Sterilisation. In order to obtain vigorous seedlings free from nitrifying and other organisms, whose presence would vitiate the results, some preliminary experiments on seed sterilisation were made. The

[1] See also Coupin, *Rév. Gén. Bot.* 1900, **12**, 177, and Suzuki, *Bull. Coll. Agric. Tokyo*, 1894—7, **2**, 265.

usual method, that of simply soaking the seeds in mercuric chloride solution, was found to be unsatisfactory owing to the persistence with which occasional air-bubbles remain on or inside the seed, and thus prevent complete sterilisation. A greater amount of success was attained by subjecting the seeds to a preliminary treatment with ether or alcohol and subsequent transference to the disinfectant solution. The most satisfactory results, however, were obtained by treating the seeds in a warm mercuric chloride solution after the removal of any air-bubbles by means of a vacuum pump; for this purpose the following apparatus was used.

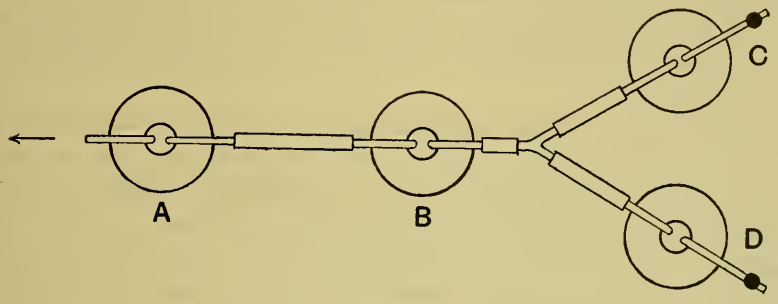

Fig. 1.

A stout-walled glass flask *B*, bearing a rubber cork with two glass tubes, was attached on the one hand to a safety flask *A*, and on the other, by means of a three-way tube, to two glass flasks of about 1 litre capacity *C* and *D*. *C* was filled with a 0·25 per cent. solution of mercuric chloride, *D* with distilled water. The whole apparatus was then sterilised in the autoclave at 125° C. for half an hour, and after being allowed to cool to 40° C., the flask *A* was attached to a vacuum pump. Seeds of approximately equal size were then placed in the flask *B* by means of a funnel—to prevent contact between the seeds and the neck of the flask—and mercuric chloride was drawn by means of the pump into *B* from *C*. The connecting tube was then closed with a screw-clip and *B* was evacuated until the solution began to boil. By this means all air-bubbles present on the surface of the seed or between the cotyledons and the seed-coat were withdrawn, and on releasing the vacuum the disinfectant solution was able to act on all portions of the seed.

Sterilisation was allowed to proceed for 3—4 minutes, and after *B* had been inverted and the disinfectant withdrawn by means of the

pump, sterilised water was allowed to flow in from *D* and the seeds well washed in 2—3 changes of water. They were then transferred to Petri dishes and a sterilised 1·25 per cent. solution of agar was poured in; solidification of the medium occurred in a few minutes, the plates were inverted and placed in the incubator at 20° C.

At the end of 3—4 days, the majority of the seeds had germinated and formed roots 1—1½ inches in length, and if sterile, remained quite free from mould or bacterial growth, and were then transferred to sterile wide glass test-tubes containing 10 c.c. distilled water over which a small plug of cotton wool had been placed. On this cotton wool the seedlings were allowed to grow until the shoot was approximately 3 inches long, and if they failed to show any subsequent infection, were then carried over to the culture bottles at the end of 7—8 days.

Culture Bottles. Many forms of apparatus have been suggested for the cultivation of plants under sterile conditions; but the majority are either too complicated or do not allow sufficient facilities for the exclusion of micro-organisms at all stages of the plant's growth. The apparatus used in these experiments has the advantage of being comparatively simple, is compact enough to allow of sterilisation in any ordinary autoclave, and may be used either for soil-, sand-, or water-cultures.

For the reception of the plant a three-necked Woulff's bottle *A* of 750—1500 c.c. capacity was taken, and rubber corks were placed in each of the side necks. One of the corks held a straight glass tube which had at its upper end a small adapter *B* filled with cotton wool, while the lower end almost touched the bottom of the bottle; this tube served to filter the air used for aerating the bottle from time to time. The other cork held a short glass tube bent at right angles which was connected to a Pasteur-Hansen flask *C*, filled with distilled water, in order that the culture solution in the Woulff's bottle could be kept to the same level throughout the course of the experiment. A few drops of concentrated sulphuric acid were placed in the side tube *D*. In many cases the flask *C* was attached to two or three Woulff's bottles by means of three- or four-way glass tubes. The middle neck of the culture bottle was firmly plugged with cotton wool and the whole apparatus heated in the steam steriliser at 99° for three hours. As soon as the sterile seedlings had formed shoots about 2½—3 inches in length they were taken from the test-tubes with sterilised forceps and the roots introduced through the middle neck of the Woulff's bottle

until they reached the culture solution; the shoot was then tightly plugged round with non-absorbent cotton wool, in order to keep the seedling in position.

Fig. 2.

Direct Assimilation of Ammonium Salts by Plants.

Series I. Wheat grown in Sand. The seeds were sterilised in 0·25 per cent. solution at 45° C. and sown on agar plates. Germination was quite normal and after 3—4 days the seedlings were transferred to sterilised test-tubes and allowed to grow for a further period of 6—7 days. On May 21st, 1908, they were carried over to 10 Woulff's bottles containing the following amounts of sand and nutrient salts.

Sand	1200 grams + 2·4 grams $CaSO_4$ + 2·4 grams $Ca_3(PO_4)_2$	
KCl	0·05 gram	dissolved in 50 c.c. distilled water
KH_2PO_4	0·10 ,,	
$MgSO_4 + 7H_2O$	0·10 ,,	
NaCl	0·05 ,,	
Fe_2Cl_6	trace	

Bottles 1—3 and 7—9 received in addition 6 grams of $CaCO_3$. The bottles and the Pasteur-Hansen flasks were sterilised in the autoclave at 125° C. for half an hour, and after cooling down a solution of ammonium sulphate = 21·98 mgms. of nitrogen was added to bottles 1—9, and sodium nitrate = 20·74 mgms. to bottle 10.

At the time of transferring the young sterile plants bottles 7—9 were inoculated with a culture of nitrifying organisms, and to all the bottles 100 c.c. distilled water was added from the Pasteur-Hansen flask. From time to time the bottles were weighed and the losses made up by adding more water, and aeration was carried out every 4—5 days.

The plants in Nos. 7—10 grew quite vigorously and possessed a dark green colour; Nos. 1—3 were also good, while 4—6 were stunted, No. 6 especially being very poor and ceasing to grow after 12—14 days. This is shown in the table by the slight amount of dry matter formed and of nitrogen assimilated.

The average amount of nitrogen in each seed was 0·71 mgm.

TABLE I. *Wheat in Sand Cultures.*

No.	Nitrogen applied	$CaCO_3$ applied		Dry matter in crop	Nitrogen total in crop	Nitrogen in dry matter
				gram	mg.	per cent.
1	Ammonium sulphate =21·98 mgms. N.	$CaCO_3$	Sterile	0·979	20·72	2·116
2	do.	do.	do.	0·882	19·72	2·236
3	do.	do.	do.	0·968	21·07	2·177
4	do.	—	do.	0·648	15·96	2·463
5	do.	—	do.	1·019	18·90	1·854
6	do.	—	do.	0·257	2·03	—
7	do.	$CaCO_3$	Inoculated with nitrifying organisms	1·325	21·70	1·638
8	do.	do.	do.	1·028	22·33	2·172
9	do.	do.	do.	1·680	21·84	1·300
10	Sodium nitrate =20·74 mgms. N.	—	Sterile	0·973	18·62	1·913

At the close of the experiment portions of the sand in each bottle were carried over to flasks containing Omelianski's solution and showed the absence of nitrifying organisms in bottles 1—6.

Series II. Wheat grown in Water Culture. This series was carried out in order to corroborate the results of the previous experiments. The treatment of the seed and seedlings was in every respect similar to that of the foregoing series, and the seedlings were transplanted when about 7 cm. high. Woulff's bottles of 850 c.cm. capacity were fitted with aeration tubes and Pasteur-Hansen flasks and were filled with the following solution:—

$MgSO_4+7H_2O$	0·5 gram	+1000 c.c. distilled water
$CaSO_4$	0·5 ,,	
KH_2PO_4	0·5 ,,	
NaCl	0·25 ,,	
KCl	0·25 ,,	
Fe_2Cl_6	10 c.c. of a 1% solution	

To Nos. 3 and 4, 5 and 6, 2 grams $CaCO_3$ was added. After the bottles had been sterilised in the autoclaves, 10 c.c. of a sterile solu-

tion of ammonium sulphate containing 21·54 mgms. N. was added, Nos. 5 and 6 were inoculated with nitrifying organisms from a liquid culture, and the sterile seedlings introduced on July 4th in a slightly etiolated condition.

From the commencement of the experiment growth in Nos. 1 and 2 was very slow, the root growth especially being very poor. During the first 3—4 weeks, Nos. 3 and 4 grew fairly rapidly and an abundance of roots was formed. These however were not equally distributed throughout the culture solution but remained in a very coiled mass near the surface of the liquid. This marked toxic effect persisted for 4—5 weeks and was subsequently followed by an even ramification of the roots in all portions of the culture solution.

On August 6th, the plants in Nos. 1—5 appeared healthy, while that in No. 6 remained etiolated for 2—3 weeks and finally died off. A marked distinction could be seen in the colour of the plants, that in No. 5 being of a much darker green than the others. From August 15th the plant in No. 4 began to grow much more vigorously, and the adoption of a darker colour seemed to indicate infection with nitrifying organisms. This would seem to be supported by the fact that both the dry matter is higher and the percentage of nitrogen lower, than in the other ammonium sulphate bottles.

Table II. *Wheat growing in Water Cultures.*

No.			Dry matter	Nitrogen total in crop	Nitrogen in dry matter
			gram	mg.	per cent.
1	No $CaCO_3$	Sterile	0·284	7·42	1·866
2	do.	do.	0·239	6·16	1·841
3	$CaCO_3$ 2 grams	do.	0·387	13·02	2·403
4	do.	do. (?)	0·872	14·28	1·169
5	do.	Inoculated with nitrifying organisms	1·208	17·50	1·035

Series III. Peas in Water Cultures. The cultures were made in Woulff's bottles holding about 1200 c.c. water in which the following amounts of the different salts were dissolved:—

	$CaSO_4$	0·5 gram
	$MgSO_4+7H_2O$	0·5 ,,
	KCl	0·25 ,,
	NaCl	0·25 ,,
	KH_2PO_4	0·5 ,,
	$(NH_4)_2SO_4$	0·389 ,,
or	$NaNO_3$	0·5 ,,
	Fe_2Cl_6	trace

The solutions were sterilised by heating for an hour at 100° on four successive days. Calcium carbonate (2 grams) was sterilised, and added to each bottle at the same time that the seedlings were put in. The bottles were arranged in sets of three, each set being connected with a Pasteur flask filled with sterilised distilled water. One set received sodium nitrate, and one ammonium sulphate, and there were two similar sets which received 2 grams of dextrose in addition, so that there were altogether twelve bottles as follows:—

I. Nos. 1, 2, 3 Sodium nitrate
II. ,, 4, 5, 6 ,, ,, +dextrose
III. ,, 7, 8, 9 Ammonium sulphate
IV. ,, 10, 11, 12 ,, ,, +dextrose

The seedlings were put in on June 1, and the plants taken up on July 20, 1908. With the exception of No. 8, which failed at an early date, all the plants grew normally and showed no appreciable differences under the different conditions. Towards the end of the experiment No. 6 suddenly lost its green colour owing to the development of a mould which quickly appropriated all the available nitrogen. All the other plants remained perfectly healthy to the end.

On taking up the plants it was found that the solutions of Nos. 3, 4, 5 and 12 were infected. The remaining ammonium solutions were free from nitrites and nitrates as well as from nitrifying organisms. In the following table are set out the amounts of dry produce, the nitrogen in the produce and in the solutions of Nos. 1, 2, 7, 9, 10 and 11.

TABLE III. *Peas growing in Water Cultures.*

No.		Dry matter	Nitrogen in dry produce	Nitrogen in plants, total	Nitrogen as NH_3 in solution	Nitrogen as N_2O_5 in solution	Total nitrogen in solution*
		grams	per cent.	gram	gram	gram	gram
1	Nitrate	3·194	2·764	0·088	0	trace	—
2	Nitrate	2·406	3·061	0·074	0	0·007	—
7	Ammonium sulphate	3·222	2·819	0·091	0·003	0	0·004
9	Ammonium sulphate	0·860	5·306	0·046	0·032	0	0·040
10	Ammonium sulphate +dextrose	2·241	3·859	0·086	0·002	0	0·007
11	Ammonium sulphate +dextrose	1·330	4·679	0·062	0·018	0	0·022

* Including any nitrogenous matter in suspension.

The small amount of growth in No. 9 is due to the failure of the original seedling; the new plant was consequently a few days behind

the others. The number of pods produced by the plants was—(1) 4, (2) 5, (7) 3, (10) 2, and (11) 2.

Additions of dextrose had no appreciable effect, probably owing to the presence in the seedlings of sufficient available non-nitrogenous material for the production of asparagine from the small amount of ammonium salt employed.

The results of the three series of experiments show that ammonium sulphate is directly assimilated by wheat and peas and that, in the case of peas, there was no difference between the plants supplied with ammonium salt and those which had sodium nitrate. The wheat plants, however, showed a decided preference for nitrogen in the form of nitrate.

Percentage of nitrogen in plants manured respectively with Ammonium Salts and Nitrates.

Reference to Tables I, II, and III, will show that in each case in which nitrogen was applied as ammonium salts, the dry matter of the plants contained higher percentages of nitrogen than when sodium nitrate was employed. Mazé (*loc. cit.*), in his water culture experiments, obtained similar indications, the percentages of nitrogen being as follows:—

Source of nitrogen	N. in dry matter
Ammonium salt.....	3·43 %
Sodium nitrate	3·17 %

Table IV. *Percentage of Nitrogen in the Mixed Herbage of the Rothamsted Grass Plots.*

Plot	Manuring	Nitrogen per cent.	
		1856—73	1901—5
14	Mixed Mineral Manure and Sodium Nitrate = 86 lb. N. per acre	*1·31	1·39
9	,, ,, Ammonium Salts = 86 ,, ,,	1·55	1·52
11	,, ,, ,, ,, = 129 ,, ,,	†1·74	1·66
5	Ammonium Salts alone ..... = 86 ,, ,,	2·16	—

* 1858—73. † 1856—1861.

Pitsch also shows (*loc. cit.*) that in the great majority of cases the ammonia plants contain higher percentages of nitrogen than the nitrate plants. Further confirmation is afforded by a comparison of the per-

centages of nitrogen in the mixed herbage from the Rothamsted grass plots, which receive their nitrogen in the form of ammonium salts and as nitrates respectively (see Table IV, p. 191).

Whilst it cannot be assumed that the whole of the nitrogen of the ammonia plots is taken up in the form of ammonia, the results as set out in the above table increase the probability that much, at any rate, of the nitrogen of the crop of plots 5, 9 and 11 is assimilated in its original form.

An explanation of the high nitrogen percentages seems to be afforded by Suzuki's results (*loc. cit.*), which showed that ammonium salts are rapidly converted by the plants into asparagine, and so give rise to conditions favourable to renewed absorption, whilst nitrates tend to accumulate and thus check further diffusion from outside. It would seem possible that the highly nitrogenous character of leguminous plants may have been acquired as a result of long continued nutrition with nitrogen, supplied from the root-nodules in a form which lends itself to more rapid production of proteids than is possible when practically the whole of the nitrogen is taken up as nitrates, as is the case with non-leguminous crops.

Conclusions.

Agricultural plants of various kinds can produce normal growth when supplied with nitrogen in the form of ammonium salts under conditions which exclude the possibility of nitrification. Some plants grow equally well with ammonium salts or nitrate as source of nitrogen. Other plants, while assimilating ammoniacal nitrogen in the absence of nitrates, appear to prefer nitrates. It is less certain whether ammonium salts can ever produce better final results than nitrates although we have indications that this may be the case.

Lehmann (17) found that whilst buckwheat failed to grow well with ammonium salts, maize did far better with this form of nitrogen than with nitrates during the first period of growth. Later on the nitrate plants recovered, and the ammonia plants became unhealthy, "ein Bild des Jammers." Kellner (19) showed that paddy rice also prefers ammonium salts to nitrates to commence with, and that nitrates are better than ammonium salts for the later growth. The best results of all were obtained when both forms of nitrogen were employed together.

Wheat plants in water-cultures with ammonium salts.

Plants which take up nitrogen exclusively in the form of ammonium salts generally contain very distinctly higher percentages of nitrogen than when supplied with nitrates. The question arises whether the high percentages of nitrogen in leguminous plants may be due to the nitrogen—or most of it—being assimilated in a form more suited to the rapid production of proteids than nitrate.

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THE DEVELOPMENT OF THE GRAIN OF WHEAT.

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It is well understood that the grain of wheat is built up out of the materials which have previously been elaborated by the plant from the crude nutriment drawn from the air and the soil and then stored in the stem, roots and leaves until the formation of the seed begins. Various observers[1] have followed out the stages in the growth of the plant and have determined the periods at which the plant ceases to draw nutriment from the soil or the air; from their investigations it would appear that during the latter part of the life of the wheat plant the manufacture of fresh material has almost ceased and that the chief process going forward is the migration of accumulated material from the stem and leaves to the grain.

For various practical reasons it is important to study this migration process in some detail and ascertain the progressive changes in the composition of the grain. For instance, it is very generally supposed that if wheat is cut in an unripe condition when the berry is still a little green, the grain will yield 'stronger' flour, *i.e.* flour capable of yielding a larger and better shaped loaf. Again, since the 'strong' wheats of commerce are in the main spring-sown wheats grown in climates which become increasingly hot and dry as the season advances, it has been supposed that a rapid growth and an accelerated ripening are factors in the production of strong wheat. If the first or the last of these suppositions are true there remains the further practical question of

[1] J. Pierre, *Mém. Soc. Linnéenne de Normandie*, xv. 1869, 1, 220; Déherain, *Ann. Agron.* viii. 1882, 23, xx. 1894, 561; J. Adorjan, *J. für Landw.* 1902, 50, 193.

how far the weight of the produce is affected if the crop is cut while still unripe or after it had experienced a premature and forced ripening.

The scientific conception which lay behind these opinions proceeded from the observation that grain contained a higher percentage of nitrogen when immature than when ripe, whereupon it was concluded that the migration of the nitrogenous materials took place first, and that during the later stages of the development little besides starch was filled into the grain. Thus grain cut unripe would contain more of the nitrogenous compounds making up the gluten, which is the chief factor in determining the strength of flour. Furthermore, if grain is rapidly grown and prematurely ripened time would not be given for the complete migration of the starch, and the grain would remain stronger because the protein has been less diluted by starch.

It has also been supposed that as the nitrogenous compounds of the grain must enter it in a soluble non-protein form, which gradually becomes converted into protein as the ripening process proceeds, another reason for the 'strength' of certain foreign wheats could be found in the thoroughness with which the conversion into protein had taken place, through the heat of the climates where they were grown.

Such are, or were, the opinions on the ripening of wheat generally accepted; their supposed basis in fact did not however prove trustworthy on experiment. For example, in the experiments of the Home Grown Wheat Committee[1] wheat cut in a green state did not yield any stronger flour than the same wheat allowed to become dead ripe; nor did variations in the date of sowing from October until March affect the strength of the resulting wheat. Moreover, from the numerous trials made by that Committee of the strength of foreign wheats grown in England and, in one case, of an English wheat grown in Hungary, it became evident that the effect of climate in determining the strength of wheat has been exaggerated. Strength turns out to be in the main a characteristic of the variety, besides which climate, soil and manuring, are only minor factors in the result. In consequence of this conflict of opinion it was decided to make a re-examination in detail of the progressive changes which could be observed in the composition and nature of the wheat grain. Not only was the migration of the materials studied by analysis but the changes in the intimate structure of the grain and of

[1] Humphries and Biffen, *J. Agri. Sci.* 1907, II. 1.

its constituent cells were followed microscopically. An account of this latter part of the work has already been published by one of us[1]; it will be sufficient here to say that no connexion could be traced between the progressive changes in the nature of the contents of the cells of the endosperm or their final structure, and the strength of the flour resulting from the grain.

The following paper deals with the chemical side of the work.

Method. In tracing the progressive changes in the migration of the materials and the filling up of the wheat grain it is necessary to ascertain the total yield on a unit area at a series of dates throughout the process, because the same plants cannot both be analysed and also allowed to grow on for analysis at a later date. This necessity at once introduces a large experimental error: even if comparatively large plots of $\frac{1}{10}$th acre, could be harvested at successive dates, the experimental error in the yield on each occasion would be not less than 10 per cent., and it is increased when the plots are reduced to the very much smaller sizes which alone are manageable in work of this kind. Errors of this kind vitiated the conclusions reached in certain earlier trials not here reported; in one year particular drills in a wheat field were selected as uniform to the eye, and on each date a fixed number of yards of corn were cut along these drills; in another year a hundred good ears were selected on each date. The results in both cases led to certain conclusions, but the experimental error was evidently too large, so the results have been discarded, though they agree with the data obtained by the more accurate methods followed in 1907 and 1908.

Certain plots of wheat were selected to provide material, and on a given day when the wheat was coming into flower all available assistants proceeded to mark by means of ties of red wool about 3000 heads of wheat which were in just the same stage of development, as shown by the fact that they had protruded one or more anthers from the middle florets of the ear. Only central stems were marked, never secondary tillered shoots; thus the work began with material as nearly as possible uniform and at the same stage of development. From among these selected shoots cuttings were made at three-day intervals; the material was brought down to the laboratory and as rapidly as possible the grain was picked from the heads. Several lots of 1000 grains were then counted out, weighed, dried and weighed again. The bulk of the grain was also dried for analysis. Finally all the analyses were calculated on the basis of the material contained in 1000 grains, this being a

[1] W. E. Brenchley, *Ann. of Botany*, Vol. 23, 1909, 117.

unit which will suffer the minimum of variation during the whole period.

In the field there will always be a good deal of variation of development between the central and the secondary shoots, hence the general produce in the field will not show the progressive changes quite so sharply as the experimental material.

In 1907 one of the wheats was selected from Plot 3 on the Broadbalk Field at Rothamsted, which had grown wheat without manure since 1843; the variety was Square Head's Master, a typical heavy-yielding weak English wheat. Though the crop on this plot is small, the grain is quite normal. Material was also taken from Plot 10 on the same field, which receives only nitrogen in the form of ammonium salts every year. The grain from this plot shows several peculiarities—it possesses a high nitrogen content and looks strong, but when a baking test of the flour is made proves to be excessively weak, though after storage for some months it gains some strength, without however reaching the normal degree for that variety. The third example was taken from the neighbouring Little Hoos Field and consisted of spring-sown Red Fife, a strong wheat of very different character from Square Head's Master. In 1908 only one wheat was selected, this was Square Head's Master grown on one of the margins of the Broadbalk Field, which had been down in grass some few years before and had also grown potatoes with farmyard manure, so that it may be taken to represent wheat grown under ordinary conditions of farming.

The actual data obtained are given in the tables in the Appendix: for purposes of discussion they have been thrown into curves, which it will be convenient to consider *seriatim* for each property determined. The Red Fife was a few days later both in flowering and cutting than the Square Head's Master, but as the march of development was quite parallel for the two varieties, the curves which follow have been drawn for corresponding periods after flowering instead of for the actual dates of sampling.

The weather conditions prevailing during the two seasons 1907 and 1908 were in marked contrast; in 1907 the summer was generally overcast and cloudy, with low temperatures and frequent rains; in 1908 the early part of the summer was hot, and though there was rain in July, August was a fine hot month up to the completion of the harvest.

The following table indicates how different was the weather in the two years:

	Rainfall		Sunshine		Temperature			
					Maximum		Minimum	
	1907	1908	1907	1908	1907	1908	1907	1908
May	2·396	1·886	164·5	198·5	59·5	63·2	42·9	46·2
June	2·609	1·675	160·1	250·8	62·5	67·9	48·4	48·4
July	2·209	2·434	170·6	205·1	65·1	69·4	48·9	51·6
August ...	1·802	*0·160	174·5	*86·7	66·6	*69·2	50·2	*50·1

* Up to Aug. 12th, the date of cutting.

Specific Gravity. In 1907 the specific gravity was determined immediately the grain had been extracted, by means of a form of volumenometer. The curves obtained in 1907 are set out in Fig. 1: they show

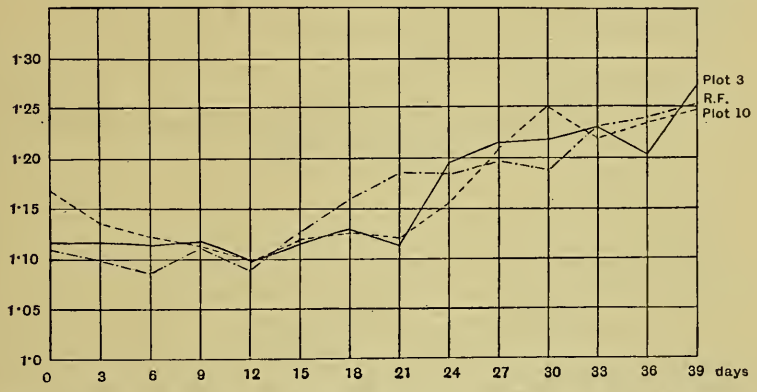


Fig. 1. Curves showing the specific gravity of the wheat grain at successive periods in 1907.

that though the experimental error is comparatively large there is evidently a slight falling off in specific gravity for the first four or five periods of three days, after which there is a continual rise up to and after the date of cutting. By combining these results with the determinations of water in the grain at each date it is possible to calculate the specific gravity of the dry matter contained in the grain, the mean curve of which for all three varieties is given in Fig. 2. From this it is evident that the specific gravity of the dry matter falls for about twelve days from the beginning of the trials, *i.e.* until the 22nd day from flowering has been reached, after which it remains constant.

Weight of Grain, Water Content, &c. Fig. 3 shows the green and dry weights respectively for each sample. The green weight rises

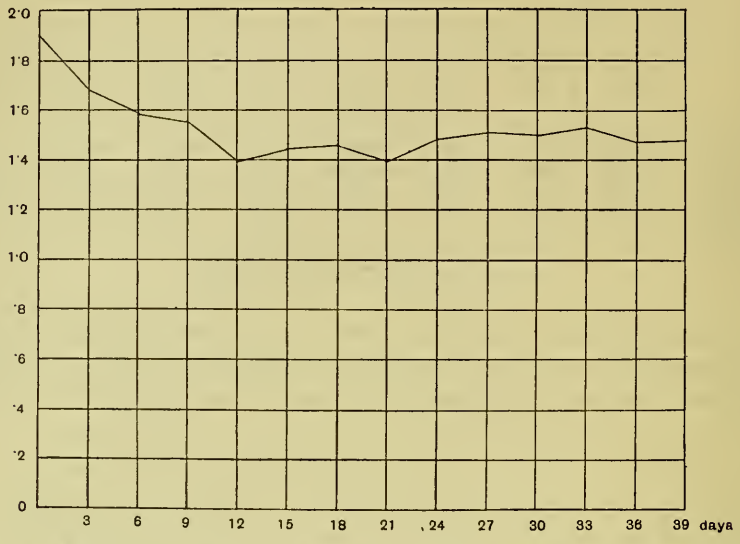


Fig. 2. Mean curve of specific gravity of dry matter contained in the grain of all three plots in 1907.

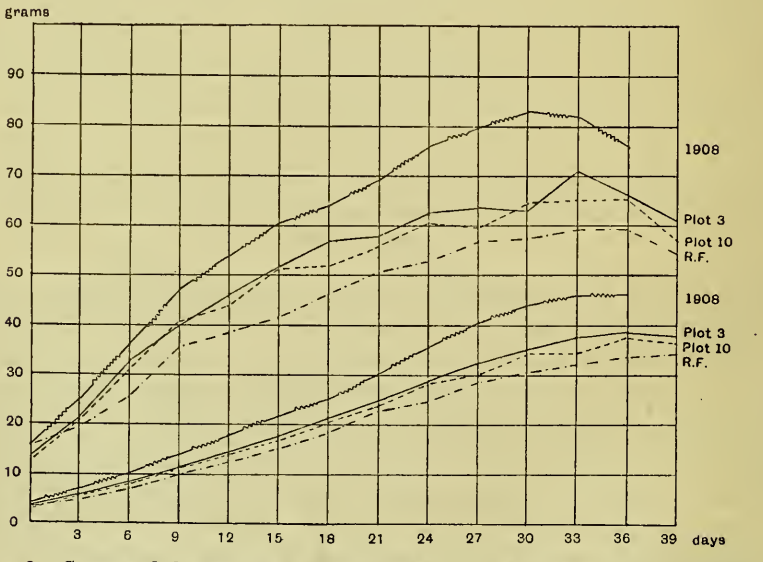


Fig. 3. Green and dry weights of 1000 grains, 1907 (3 plots) and 1908 (1 plot). Upper set of curves represent green weight, lower set dry weight.

steadily until about six days before cutting, after which it falls off: the dry weight rises steadily, though there is little increase in the last six days. The riper Square Head's Master even shows a slight but perceptible decrease in the weight of 1000 grains in the last three days. This is probably real and due to the continuance of respiration after migration had ceased, though the loss is so small that it falls within the limits of experimental error. It is impossible to obtain quite concordant results in drying material like grain, which will continue to lose water in the drying oven at 100° C. for an indefinite period.

Fig. 4 shows the relationship of green to dry weight—all three samples in 1907 follow a very parallel course, the notable features of

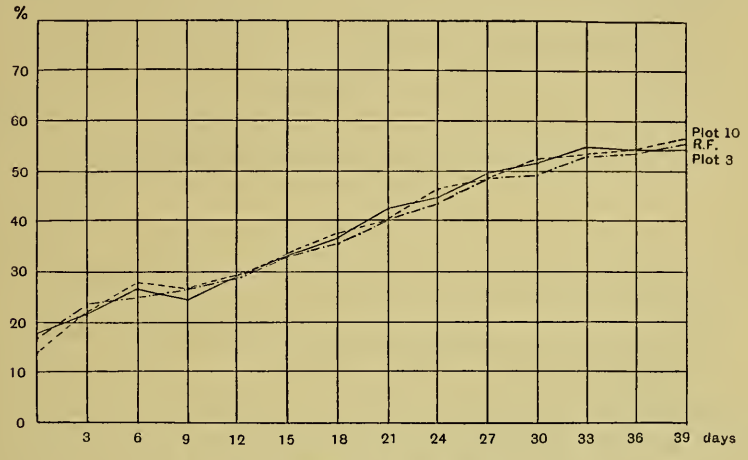


Fig. 4. % dry weight to green weight, 1907 only.

which are a change of curvature after the third period, and another change about six or nine days before cutting. Both these breaks are symptomatic; as will be seen later the first marks the final contraction and drying up of the pericarp, the second indicates the beginning of desiccation and the conclusion of the migration.

Fig. 5 shows the actual water contained in 1000 grains and is highly instructive: the water rises until the third or fourth period, then it remains approximately constant in amount until six days from cutting, after which it falls rapidly. Again the two critical dates are about twelve days after the first sampling and six days before cutting.

Nitrogen. The percentage of nitrogen in the dry matter of the grain (Fig. 6) falls rapidly at first but after the first six periods becomes

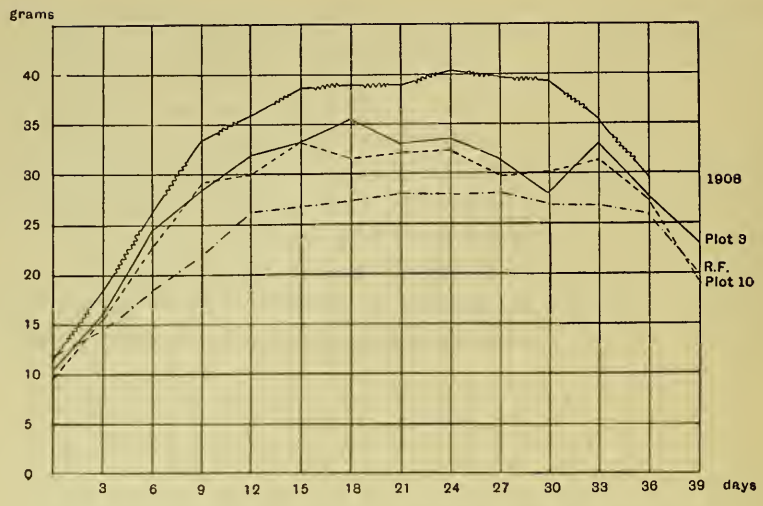


Fig. 5. Actual water contained in 1000 grains, 1907 and 1908.

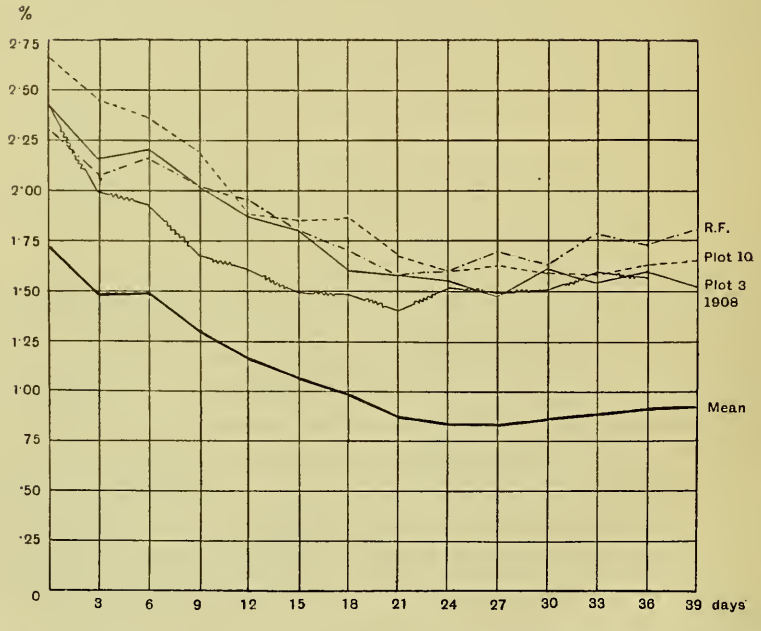


Fig. 6. °/₀ nitrogen in dry matter of grain, 1907 and 1908. The dark line shows the mean curve of the three plots for 1907, and is placed three squares too low for the sake of clearness.

fairly constant: there is some indication of a rise towards the end, but the curves are not smooth enough to be sure of this, though as will be seen later it is explicable by the continued loss of non-nitrogenous matter by respiration. The actual nitrogen in 1000 grains (Fig. 7)

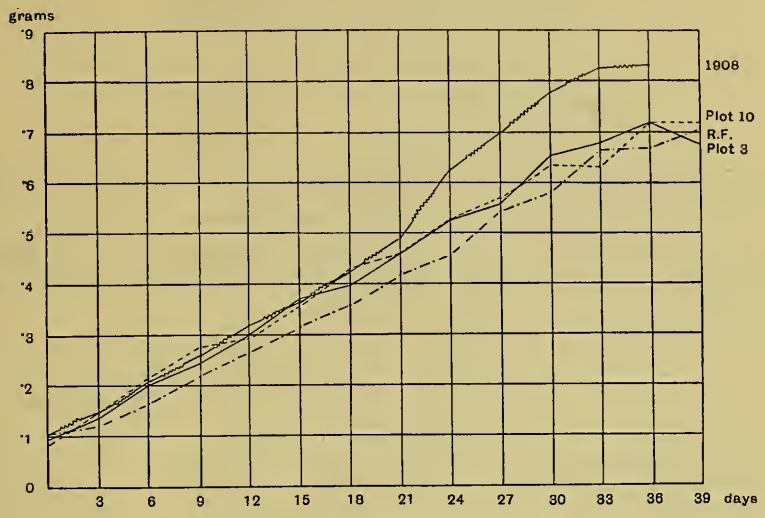


Fig. 7. Actual nitrogen contained in 1000 grains, 1907 and 1908.

rises regularly until the last three-day period. The very steady increment of nitrogen in itself disposes of the opinion that the nitrogenous constituents enter the endosperm first, and that the later filling of the grain consists mainly of starch. Confirmation is obtained by recalculating the results so as to ascertain the proportion of nitrogen in the dry matter that has entered the grain between successive dates, though the figures obtained can only be viewed very generally, because the experimental errors are accumulated in quantities that are not themselves large. Comparing, however, the first and second halves of the whole period we get the following proportions of nitrogen in the dry matter.

Percentages of Nitrogen in Dry Matter entering the Grain.

Plot 3.

July 19—August 6	1·667
August 6—24	1·681

Plot 10.

July 16—August 3 ..... 1·698
August 3—21 ..... 1·868

Red Fife, 1907.

July 25—August 12 ..... 1·692
August 12—30 ..... 2·452

Square Head's Master, 1908.

July 3—21 ..... 1·552
July 21—August 8..... 1·912

These figures show that the material filled into the grain is more nitrogenous in the later than in the earlier stages.

A better idea of what takes place may be obtained by dividing the whole period into three stages suggested by the variations in the water in the grain.

	Plot 3	Plot 10	Red Fife	Square Head's Master, 1908
Stage of increasing water ...	1·932	1·916	2·035	1·612
Stage of constant water.....	1·592	1·631	1·930	1·677
Stage of desiccation	?	2·700	2·415	1·989

In the first stage the larger part of the grain consists of the soft tissue forming the pericarp, the subsequent shrinkage of which into dry membranes is practically complete by the end of the first stage. The endosperm exists during all the first stage and at the end is beginning to show starch, &c. throughout. The material forming the pericarp evidently contains more nitrogen than that which enters the endosperm later. The middle stage is characterised by the filling in of the endosperm, and in the last stage the migration is coming to an end; during this period the material that is stored appears to be more nitrogenous because the entry is slow, while the losses by respiration, which fall wholly on the non-nitrogenous substance, are still going on.

Ash and Phosphoric Acid. The proportion of ash in dry matter, and the amounts of ash and of phosphoric acid in 1000 grains, yield curves exactly similar to those given by nitrogen, indicating that the ash and the phosphoric acid enter the grain *pari passu* with the nitrogen. Table I. shows the ratio of phosphoric acid to nitrogen for each sample, and indicates that the wheat on each plot manufactures material possessing a composition special to itself, but one which remains

approximately constant during the whole formation of the grain. Similar constant ratios are obtained between the nitrogen, phosphoric acid and carbohydrates, the carbohydrates being reckoned as dry matter less protein and ash.

Table I. $\frac{Nitrogen}{Phosphoric\ acid}$. *Ratio.*

Days	Plot 3	Plot 10	Red Fife	Square Head's Master, 1908
0	2·150	—	—	2·170
3	2·154	—	—	1·945
6	2·122	2·351	—	1·950
9	1·995	2·323	1·907	1·839
12	2·051	2·253	1·877	1·717
15	2·220	2·528	1·909	1·687
18	1·869	2·315	1·850	1·690
21	2·035	2·268	1·819	1·643
24	1·890	2·503	2·000	1·848
27	1·859	2·453	1·805	1·843
30	2·279	2·409	1·821	1·786
33	1·963	2·167	1·909	1·826
36	2·064	2·294	1·851	1·811
39	1·987	2·375	1·977	—

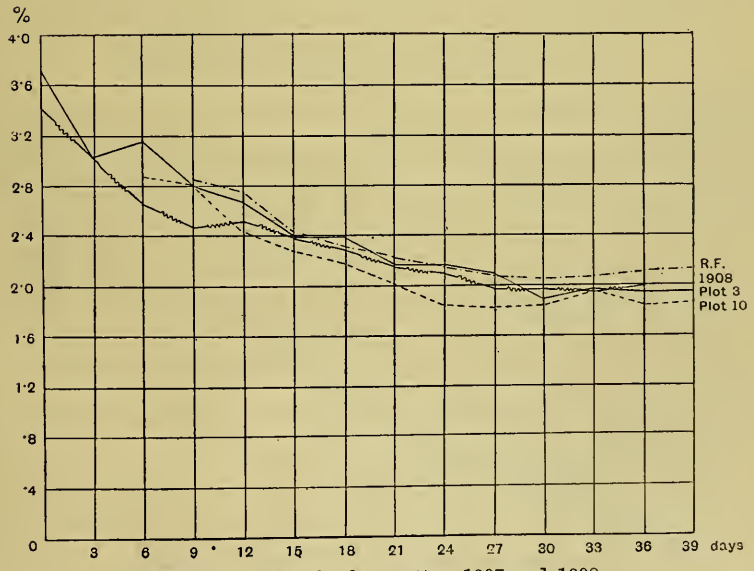


Fig. 8. % ash in dry matter, 1907 and 1908.

It may be noted here that when wheats from different plots, &c. are compared, there is no connexion between the actual percentage of

nitrogen in the grain and the nitrogen-phosphoric acid ratio. It has often been supposed that the extent to which the plant can utilise nitrogen in the soil is dependent upon the phosphoric acid also present, because the phosphoric acid acts in some way as a carrier of nitrogen,

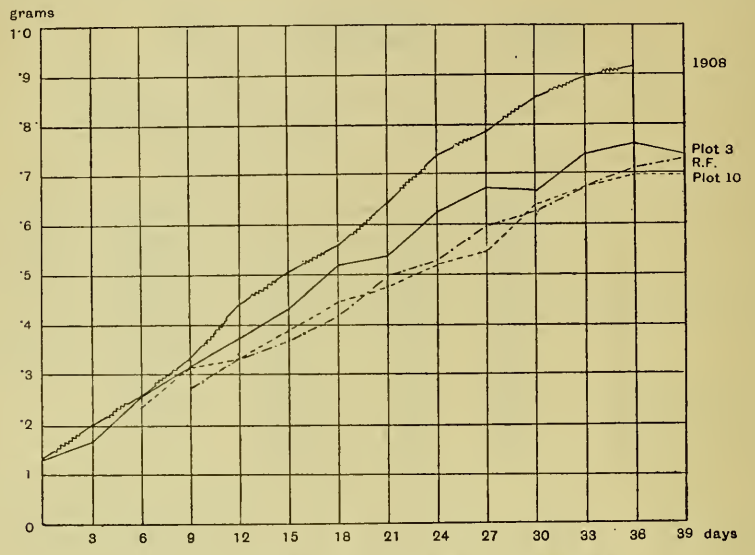


Fig. 9. Actual ash in 1000 grains, 1907 and 1908.

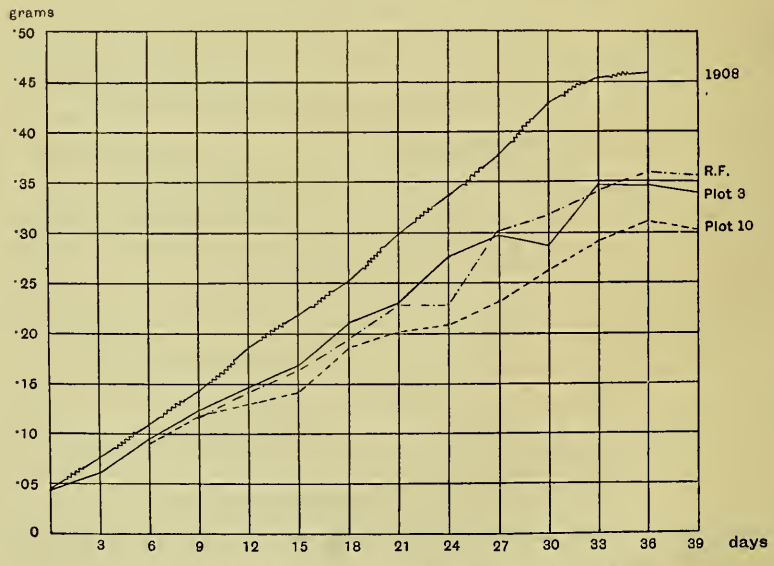


Fig. 10. Actual P_2O_5 in 1000 grains, 1907 and 1908.

but the wheat from plot 10 is exceptionally nitrogenous for the variety, and at the same time exceptionally poor in phosphoric acid.

Other determinations. A certain number of determinations were made in order to asertain if any marked change could be found in the nature of the materials accumulated in the grain, by which the degree of ripeness could be gauged. Fig. 11 shows the percentage of sugar

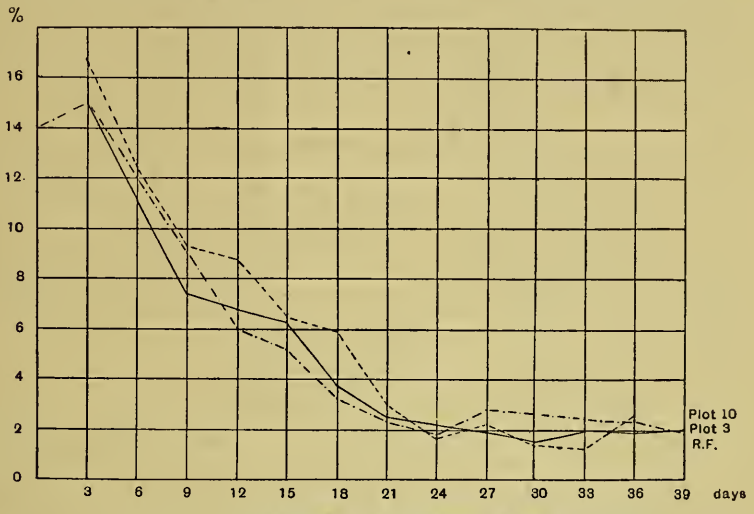


Fig. 11. % dextrose in dry matter of grain, 1907.

(dextrose) in the dry matter of the grain: it is high at first, about 15 per cent., but falls rapidly to about 2 per cent., at which figure it keeps fairly constant for the last fortnight before cutting. On recalculating to ascertain the amount of dextrose per 1000 grains (Fig. 12)

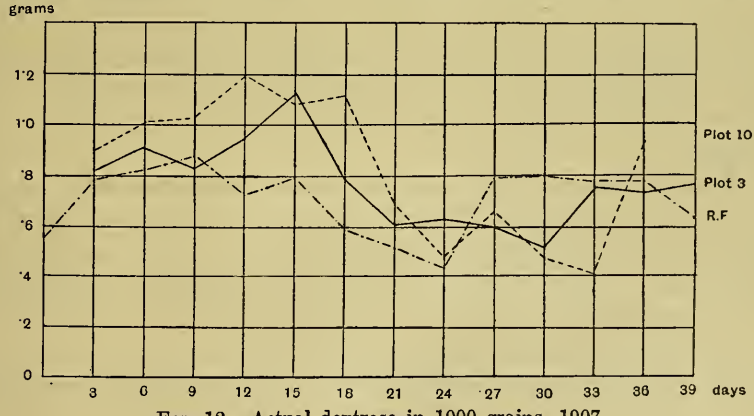


Fig. 12. Actual dextrose in 1000 grains, 1907.

it is seen to increase for the first three or four periods (*i.e.* while the living tissues of the pericarp form the most prominent feature in the grain), then it falls rapidly, and during the last fortnight it remains approximately constant, though the figures are evidently affected by a large experimental error.

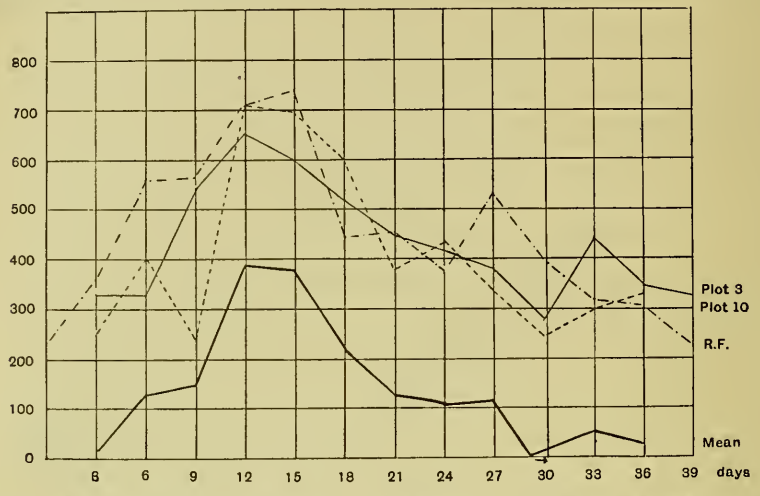


Fig. 13. Maltose produced per 100 of dry matter, 1907. The dark line shows the mean curve for the three plots, placed three squares too low for the sake of clearness.

Determinations of the diastatic power were made by rapidly macerating the fresh grain and adding it to starch paste: Fig. 13 shows the amount of maltose thus produced per 100 of dry matter in the grain. The results are subject to a large experimental error, but indicate that the diastatic power of the material, taken as a whole, rises during the first four or five periods and then falls steadily. Again recalculating the results to show diastatic power per 1000 grains (Fig. 14), this property rises for five periods and then probably remains constant.

Owing to an accident only one set of determinations of protein nitrogen are available, for 1908; these show (Fig. 15) a marked rise in the proportion of nitrogen in the protein form during the period of experiment. At first about 72 per cent. of the nitrogen is combined as protein but this gradually rises to over 99 per cent.

On the same figure is shown the actual amount of non-protein nitrogen contained in 1000 grains; it rises at first, then remains approximately constant, and finally falls rapidly during the last desic-

cation stage. Evidently the end process of ripening is accompanied by a change from non-protein to protein nitrogenous compounds.

General outline of the process of migration. It is now possible to summarise the whole process of the migration of the reserve materials into the wheat grain. The first samples were taken about ten days after flowering; at this time the endosperm is just formed, but the grain is in the main made up of the active living tissues constituting the pericarp. The figures for July 14th in Plate XV (taken from W. E. Brenchley, *loc. cit.*) show the structure of the grain at this stage. During the

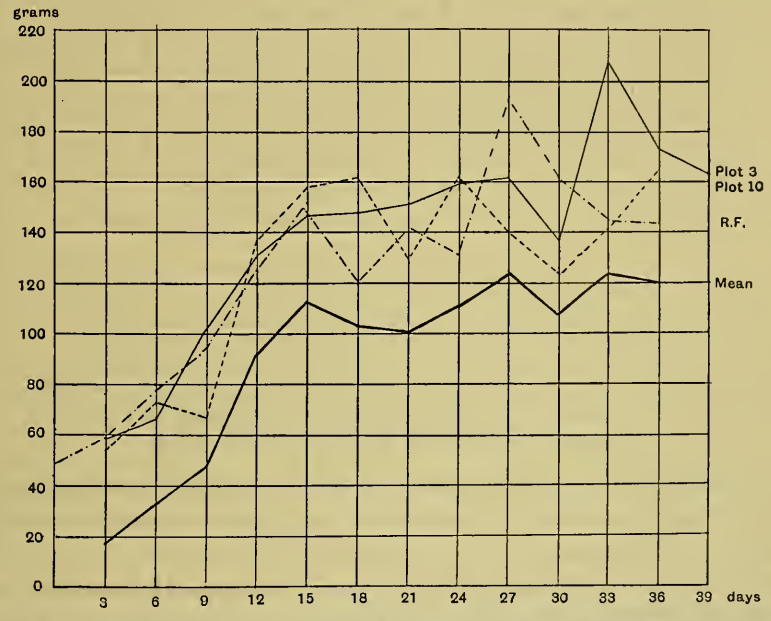


Fig. 14. Maltose produced per 1000 grains, 1907. The dark line shows the mean curve of the three plots, placed two squares too low for the sake of clearness.

next twelve days the endosperm is beginning to fill, as shown by the appearance of starch grains in the cells, until by the end of the period starch is to be found throughout the endosperm. But the most characteristic feature of this stage is the depletion of the cells in the pericarp and their crushing together, until they become nothing more than membranes containing no living cells; the end of this stage being shown by the second set of figures in the plate. It is during this period that the nitrogen percentage of the grain is falling rapidly; the cells of the pericarp when active evidently possess a comparatively high

proportion of nitrogen and ash, though the percentage of phosphoric acid in the ash is low. Both the dextrose and the diastatic power of the grain are rising during this period.

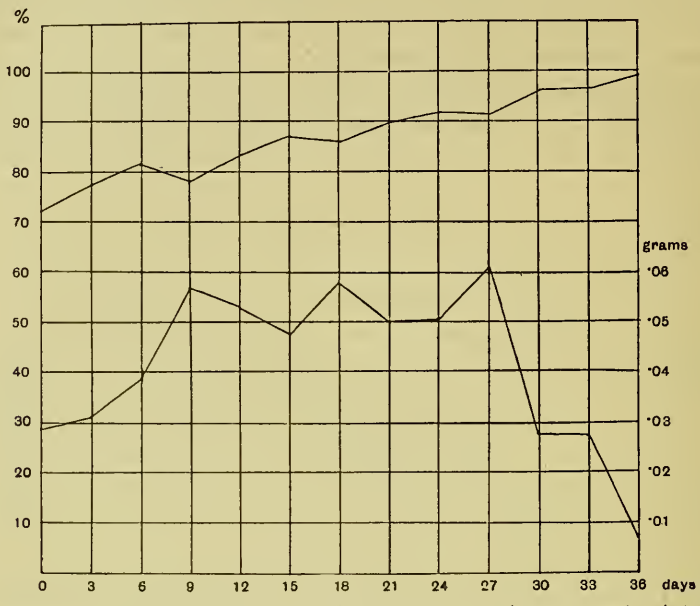


Fig. 15. % protein nitrogen in total nitrogen, 1908 (upper curve). Actual non-protein nitrogen in 1000 grains (lower curve).

During the next period, which lasts about a month, the endosperm is being filled up, and the dry weight of the grain is more than trebled, but the actual amount of water present in the grain remains approximately constant. Throughout this time the material moved by the plant and stored in the endosperm appears to be of constant composition, as indicated by the uniformity of the N : P_2O_5 : carbohydrate ratio of the material entering between successive dates. Each wheat however elaborates and stores a characteristic material, the composition of which is determined beforehand by variety, soil (including manure), climate, and similar factors independent of the migration process. The microscopic examination of the grain would show that the cells of the endosperm are filled progressively beginning from the base of the grain and proceeding towards the tip, or end at which the embryo is developed, each set of cells being successively filled up and then as it were put out of action. The fact that the total amount of water, non-protein nitrogen, diastatic power, and dextrose (though this latter material does not become constant

until a later period than the others) remain constant during the filling stage, indicates that these materials belong to the active cells which are being filled, rather than to the cells which have been filled up and put out of action.

Finally ripening begins about six days before cutting, and the characteristic feature is the rapid desiccation of the grain; the actual water falls as the remaining active cells fill up, the non-protein nitrogen drops, and the precentage of nitrogen in the material still entering increases, because the losses by respiration overtake the gain by migration. The maximum weight of dry matter is reached a few days before the grain appears to be ripe for cutting, because the intake ceases, while respiration still continues. Cytologically this last stage of ripening is marked by the progressive destruction of the nuclei in the endosperm as they are squeezed into networks by the pressure of the starch grains, but no sequence can be traced in the regions showing such deformed nuclei, such as was observed by Brown and Escombe in barley, which shows a progressive 'nuclear senescence' with ripening.

Relation of the migration process to the nutrition of the whole plant. Since the publication of Pierre's investigations (*loc. cit.*) it has been generally held that the wheat plant ceases to draw nutriment from the soil after a comparatively early date—the flowering period or a little later. Assimilation, however, was considered to go on later, but to cease in its turn before the migration into the grain had been completed; it has even been held that there is a return of nutrient materials to the soil, an actual excretion of phosphoric acid, nitrogen, &c. in the final stages. Such a complete cessation of nutrition and assimilation must however be a matter of season and climate; as long as any part of the plant remains green assimilation will go on, water will be drawn from the soil, and with the transpiration current nutrient materials will enter the plant. In 1908 the straw belonging to each of the marked ears was cut off close to the ground and analysed in order to trace the relationship between migration and the nutrition of the whole plant. The ratio between grain and straw in these selected shoots was determined and as before the unit yield at each date is represented by the material contained in 1000 grains and also in the straw which was found to be associated with 1000 grains at that period.

Fig. 16 shows the dry matter curves for the whole plant and for the grain; from which it will be seen that the dry weight of the whole plant increases up to within a week of cutting, *i.e.* the point when desiccation

in the grain sets in. It is evident that assimilation does not cease until migration is nearly complete. Respiration continues later still, because the weight of the whole plant falls in the last week.

Fig. 17 shows the nitrogen in the whole plant and in the grain; here again, though the curve is not very smooth, there is no evidence of any cessation in the intake of nitrogen until within a few days of the date of cutting.

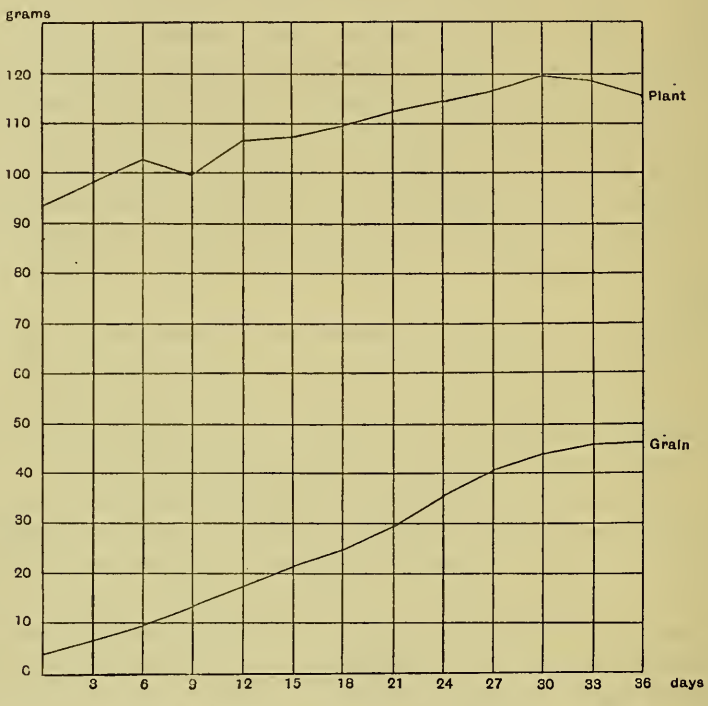


Fig. 16. Dry weights of whole plant and grain, 1908 (whole plant = weight of 1000 grains + weight of straw calculated as equivalent to 1000 grains).

Fig. 18 shows the ash in the whole plant and in the grain; similarly it is seen that the intake of ash by the plant, though not so pronounced during the period under review, continues to within a week of cutting. The amount of ash then becomes stationary, the slight fall indicated in the last week being within the limits of experimental error. Exactly similar conclusions are to be drawn from the phosphoric acid curves set out in Fig. 19.

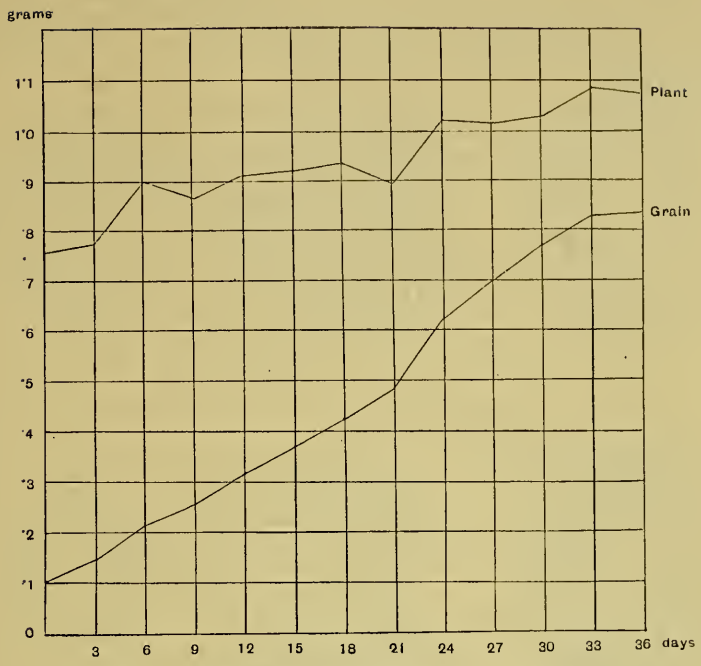


Fig. 17. Nitrogen in whole plant and in grain, 1908.

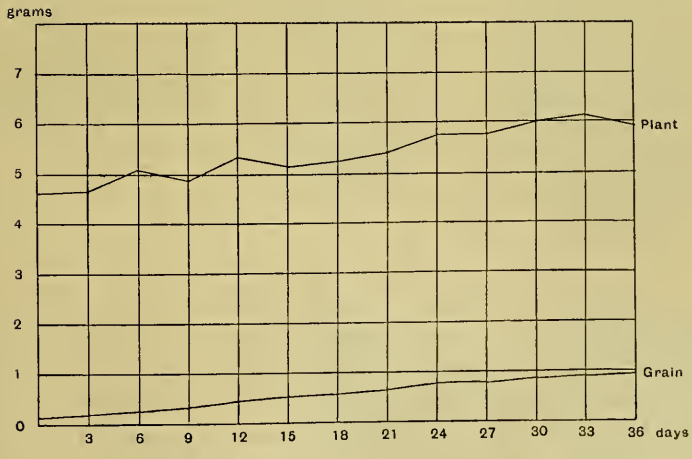


Fig. 18. Ash in whole plant and in grain, 1908.

Fig. 20, showing the percentage of nitrogen and phosphoric acid in the straw, has been drawn in order to demonstrate how the feeding value of the straw declines as the grain forms.

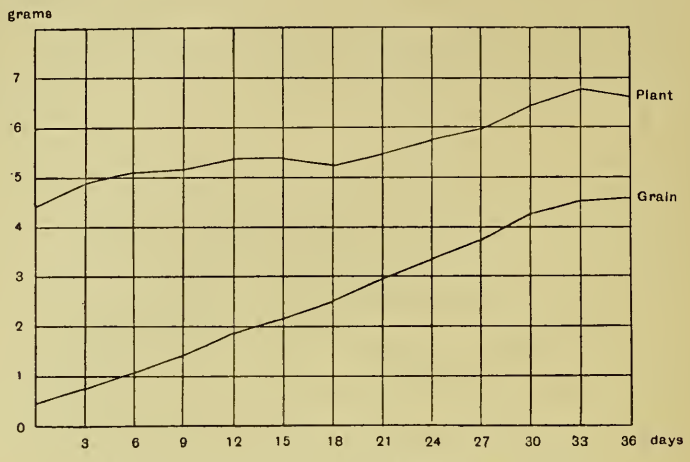


Fig. 19. P_2O_5 in whole plant and in grain, 1908.

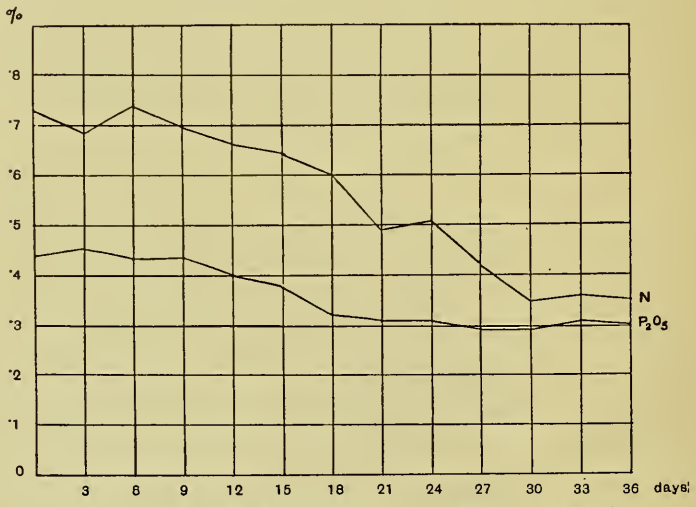
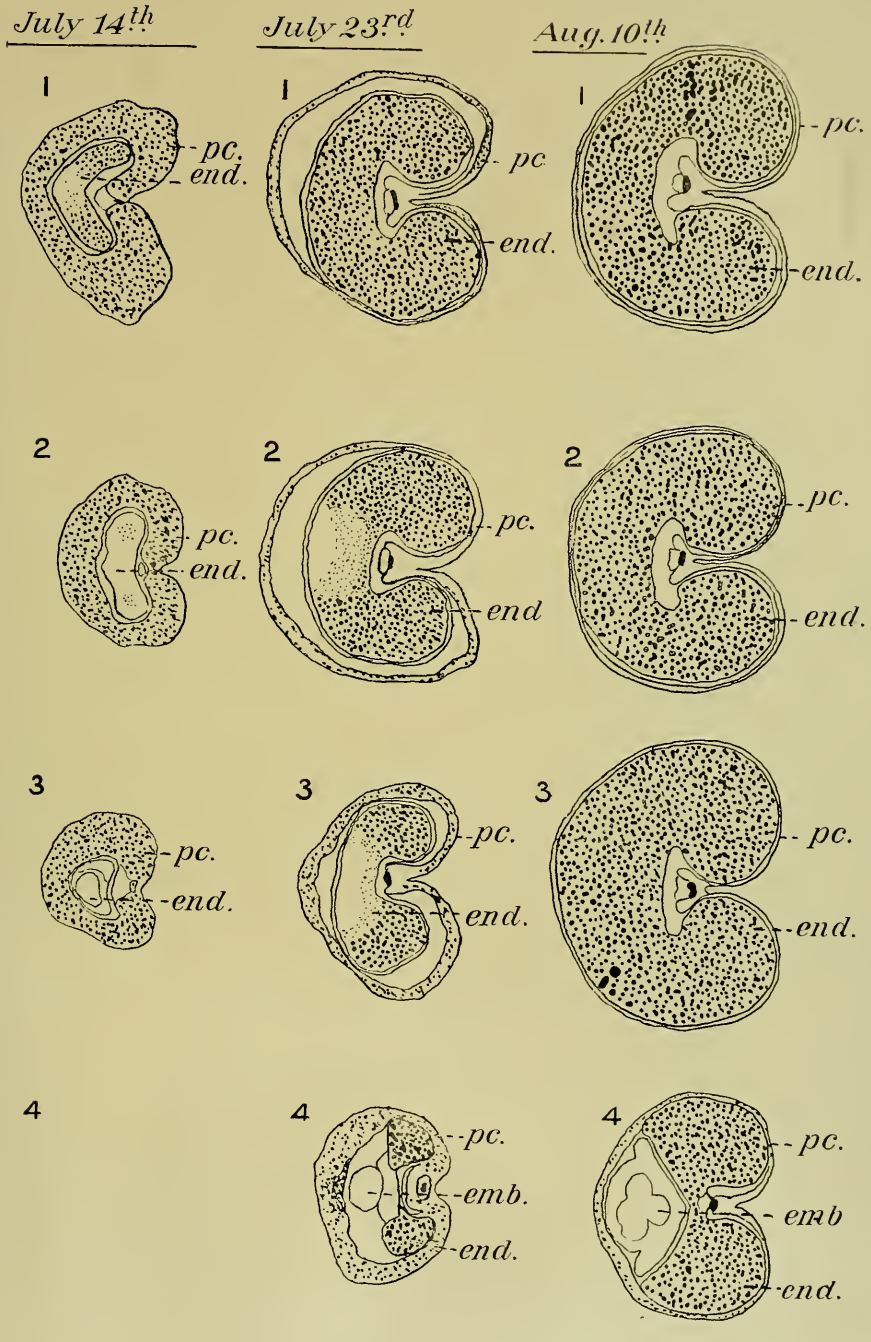


Fig. 20. % nitrogen and P_2O_5 in dry matter of straw, 1908.

It should of course be remembered that in these results no account is taken of the root of the plant, which cannot be removed from the soil without both loss of the fine roots and the introduction of foreign matter; migration will no doubt take place from the root to the seed, but the weight of root bears too small a proportion to that of the


July 14th
July 23rd
Aug. 10th
1
pc.
end.
2
3
4
emb.

whole plant to account for the rise in dry matter, &c. that is observed in the grain and straw during the migration period. The question of when nutrition and assimilation finally cease can only be definitely settled when the roots also can be examined, and experiments to that end are now in progress. Meantime the evidence derived from our experiments is against the view that either nutrition or assimilation ceases before the final ripening off of the wheat grain; this, however, may only be true for the comparatively humid English climate where the wheat plant retains some green active tissue until harvest is close at hand.

Summary.

A study during 1907 and 1908 of various plots of wheat cut at three-day intervals leads to the following general conclusions:

(1) The whole plant, and with it the nitrogen, ash, and phosphoric acid it contains, increases in weight until about a week before it would be regarded as ready to cut. Some decrease of dry weight takes place during the last week.

(2) In the formation of the grain three stages may be distinguished:

(*a*) a period during which the pericarp is the most prominent feature,

(*b*) the main period during which the endosperm is filled,

(*c*) the ripening period characterised by the desiccation of the grain.

(3) For the filling of the endosperm each plant possesses as it were a special mould, and continually moves into the grain uniform material cast in that mould, possessing always the same ratio of nitrogenous to non-nitrogenous materials and ash. The character of the mould possessed by each plant is determined by variety, soil, season, &c.

(4) The main feature of the ripening process is desiccation rather than the setting in of such chemical changes as the conversion of sugars into starch, non-protein into protein, though the latter change also takes place.

(5) The maximum dry weight of grain is attained a day or two before the grain would be regarded as ripe by the farmer. Allowing for the fact that the tillered shoots are a little behind the central shoots, no loss of weight in the crop will be incurred by cutting before the corn appears quite ripe, while a number of accidental mechanical losses due to birds, shedding, weather, may thus be avoided. Other experiments have shown that, though there may be no gain, there will be no loss in the quality of the wheat due to such early cutting.

Appendix I.

Broadbalk, Plot 3, 1907.

Date	Green weight of 1000 grains	Dry weight of 1000 grains	Specific gravity	% nitrogen in dry matter	% ash in dry matter	% P_2O_5 in ash	% dextrose in dry matter	Maltose produced per 100 of dry matter
	grams	grams						
July 16	13·75	3·51	1·116	2·679	3·70	33·66	—	—
,, 19	21·05	5·43	1·116	2·406	3·03	36·91	14·99	339·4
,, 22	32·47	8·14	1·113	2·458	3·14	36·88	11·08	324·7
,, 25	39·70	11·16	1·116	2·167	2·80	38·73	7·36	541·4
,, 28	45·95	14·05	1·099	2·119	2·66	38·86	6·71	650·7
,, 31	51·30	17·99	1·116	2·055	2·39	38·68	6·23	597·1
Aug. 3	56·69	21·15	1·128	1·856	2·38	40·35	3·70	510·6
,, 6	57·91	24·97	1·113	1·828	2·16	42·54	2·42	442·9
,, 9	62·48	28·98	1·196	1·801	2·16	44·17	2·17	412·0
,, 12	63·68	32·20	1·215	1·720	2·09	44·30	1·86	378·1
,, 15	63·19	35·09	1·218	1·856	1·89	44·06	1·46	277·9
,, 18	70·89	37·93	1·231	1·787	1·96	46·50	1·99	441·6
,, 21	66·30	38·69	1·204	1·846	1·94	46·13	1·91	343·7
,, 24	61·01	37·96	1·271	1·778	1·93	46·30	2·02	322·1

Broadbalk, Plot 10, 1907.

July 13	12·45	2·93	1·169	2·910	—	—	—	—
,, 16	20·92	5·36	1·136	2·694	—	—	16·76	248·6
,, 19	30·81	8·13	1·122	2·611	2·87	38·64	12·42	404·7
,, 22	40·21	11·16	1·114	2·445	2·80	37·62	9·24	235·7
,, 25	43·59	13·69	1·098	2·128	2·43	38·91	8·73	701·4
,, 28	50·19	16·95	1·119	2·100	2·28	39·11	6·41	694·1
,, 31	51·85	20·34	1·125	2·113	2·18	41·72	5·84	597·0
Aug. 3	55·70	23·66	1·120	1·923	2·01	42·24	2·97	376·1
,, 6	60·43	28·23	1·153	1·845	1·84	40·07	1·66	430·7
,, 9	59·99	30·10	1·208	1·877	1·81	42·22	2·18	332·4
,, 12	64·47	34·45	1·251	1·832	1·85	41·08	1·38	242·2
,, 15	65·65	34·45	1·221	1·829	1·96	43·18	1·17	294·8
,, 18	65·29	37·93	1·236	1·875	1·84	44·35	2·48	327·6
,, 21	56·93	37·65	1·249	1·903	1·86	43·19	—	—

Red Fife, 1907.

July 22	15·66	3·89	1·110	2·552	—	38·42	13·98	230·9
,, 25	19·64	5·17	1·098	2·322	—	40·13	14·93	358·9
,, 28	25·17	6·79	1·085	2·404	—	42·59	11·98	556·3
,, 31	31·54	9·58	1·112	2·271	2·839	41·94	9·13	563·9
Aug. 3	38·20	12·01	1·088	2·202	2·749	42·69	6·00	705·2
,, 6	41·86	15·17	1·124	2·049	2·422	44·29	5·17	735·9
,, 9	46·05	18·18	1·157	1·967	2·291	46·41	3·21	440·5
,, 12	50·55	22·49	1·185	1·837	2·201	45·87	2·27	451·1
,, 15	52·47	24·63	1·184	1·848	2·151	46·04	1·73	372·2
,, 18	56·73	28·68	1·197	1·886	2·075	50·34	2·75	529·3
,, 21	57·52	30·75	1·187	1·879	2·046	50·43	2·60	394·3
,, 24	59·40	32·61	1·231	2·030	2·075	51·26	2·39	316·6
,, 27	59·33	33·58	1·238	1·979	2·117	50·52	2·32	307·1
,, 30	54·48	34·33	1·254	2·050	2·133	48·62	1·82	218·5

Square Head's Master, 1908.

Date	Green weight of 1000 grains	Dry weight of 1000 grains	% nitrogen in dry matter. Grain	% protein N. in total nitrogen. Grain	% nitrogen in dry matter. Straw	% ash in dry matter		% P_2O_5 in ash		Grain to Straw (=100) ratio dry weights
						Grain	Straw	Grain	Straw	
	grams	grams								
July 3	15·13	3·81	2·676	72·13	·731	3·42	5·00	36·08	8·80	4·24
,, 6	24·78	6·55	2·245	77·50	·684	3·04	4·89	38·03	9·26	7·16
,, 9	35·82	9·73	2·177	81·79	·736	2·64	5·18	42·22	8·29	10·46
,, 12	46·51	13·44	1·926	78·09	·699	2·46	5·24	42·62	8·24	15·56
,, 15	53·20	17·26	1·846	83·48	·663	2·53	5·48	42·54	7·26	19·41
,, 18	59·74	21·13	1·737	87·12	·642	2·37	5·38	43·40	6·92	24·56
,, 21	63·41	24·49	1·727	86·36	·599	2·28	5·57	44·73	5·73	28·90
,, 24	68·61	29·67	1·643	89·73	·488	2·16	5·79	46·33	5·24	35·91
,, 27	75·54	35·24	1·760	91·91	·505	2·09	6·35	45·54	4·83	44·63
,, 30	79·38	40·06	1·733	91·27	·420	1·96	6·55	47·91	4·43	52·46
Aug. 2	82·79	43·73	1·754	96·42	·343	1·96	6·91	50·11	4·18	57·92
,, 5	81·26	45·88	1·801	96·72	·356	1·95	7·28	50·50	4·25	63·54
,, 8	75·61	45·83	1·813	99·13	·348	2·00	7·19	50·18	4·07	66·03

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